

Mosaic trisomy 2 and trisomy 11 in myelodysplastic syndrome (MDS). *M. Gustavo¹, M. DaCosta¹, V. Mizhiritskaya², M.J. Macera², R.M. Chandra², R.S. Verma^{1,2}.* 1) Department of Medicine, SUNY Health Science Center, Brooklyn, NY; 2) Department of Medicine, Wyckoff Heights Medical Center, Brooklyn-New York Hospital/Weill Medical College of Cornell Medical Center, New York, NY.

DH is an 86 year-old African-American woman who came with fatigue which continued to worsen. She has a long history of hypertension and was treated with Ca++ channel blocker. Her past medical history included colonic polyps with rectal bleeding complicated by cardiac arrest during colonoscopy 5 years ago, a hysterectomy for uterine fibroids 30 years ago and spinal disc surgery 25 years ago. The peripheral blood smear showed about 15% of blasts that morphologically belong to the myeloid series. A CBC was as follows: WBC 1.63 k/ml, HGB 4.5 gm/dl, HCT 13.8%, MCV 111 fl and platelets were 65 k/ml. The bone marrow aspirate showed diffuse infiltration with blast cells with prominent nucleoli. A few normal erythroid and myeloid cells were scattered among the immature blasts. By flow cytometry the blasts in the peripheral blood were positive for CD33 and CD13 and negative for B- and T- cell markers. Cytogenetic findings with G-banding revealed 17 cells with 47,XX,+11 and three cells with 47,XX,+2 i.e. 47,XX,+11[17]/47,XX,+2[3]. The FISH technique using alphoid centromeric probes for chromosome 2 and 11 revealed 69 nuclei with an additional chromosome 11, six cells with trisomy 2 and 48 normal cells. By interphase cytogenetics, we were able to identify a normal clone. Though on initial presentation, the peripheral blood and bone marrow morphology was that of de novo AML, the recovery of the platelet count and the presence of karyotypically normal cells in the bone marrow suggest that this patient may have a myelodysplastic syndrome in transition to AML. Although trisomy 11 is a known cytogenetic abnormality in AML, the presence of an additional clone with trisomy 2 has not been described and its molecular pathogenesis and prognostic significance are unknown.

New cytogenetic findings in acute myelomonocytic leukemia. *F.P. Chaudhri¹, P. Hegde¹, N.C. Shah¹, V. Mizhiritskaya², M.J. Macera², R.S. Verma².* 1) Department of Medicine, Flushing Hospital Medical Center, Flushing, NY; 2) Department of Medicine, Wyckoff Heights Medical Center, Brooklyn-NY Hospital/Weill Medical College of Cornell University, New York, N.Y.

The diagnosis of acute myelomonocytic leukemia [AMMoL] based on hematologic features alone has been an arduous task. Although the cytogenetic findings remain inconclusive, the inversion or deletion of chromosome 16 are the most frequent and consistent genetic changes. Other changes with a relatively high incidence are trisomy 8, monosomy 7, 11q-, and t(1;11). A 75 year-old male with cytopenia and chronic leukemia was referred for evaluation. CBC revealed: WBC 13.6, Hb 9.3, Hct 27.7, HCV 105 and plat 95,000. The differentials were 7% segmented neutrophils and bands, 28% lymphocytes, 62% monocytes and pro-monocytes and 3% blasts. Based on the aforementioned findings, a diagnosis of CMMoL was suspected. Bone marrow aspirate revealed 77% blasts (myeloid and monocytic). Immunophenotyping revealed an immature myelomonocytic population with the following immunophenotype: HLA-DR+/CD15+/CD33+/CD4+/CD13+/CD34/CD11c/CD14 (greater than 20%). All those changes are consistent with AMMoL [FAB-M4]. Cytochemistry revealed myeloperoxidase positive, and alpha-naphthyl butyrate esterase and chloroacetate esterase both greater than 20%. Cytogenetic analysis using G-banding revealed an abnormal 45,XY,t(7;17)(p11.1;q11.2). Molecular analysis using WCP painting probe for chromosomes 7 and 17 revealed 47XY,t(7;17)(p11.1;q11.2).ish(wcp17+,wcp7+;wcp17-,wcp7-). The loss of the short arms of both chromosome 7 and 17 during translocation is a significant finding. As such, the complete monosomy of chromosome 7 may not be a factor for the development of this malignancy and these new findings may be of interest to hematologists.

Chromosomal basis of renal cell carcinoma. *S.M Bahieg*¹, *R.S Verma*^{1,2}. 1) Div. of Molecular Med. & Gen., Wyckoff Heights Medical Center, Brooklyn, NY; 2) New York Hospital/Weill Medical College of Cornell Medical Center, New York, N.Y.

Renal cell carcinoma [RCC] is the most common cancer in the kidney affecting over 30,000 new cases with an annual mortality of 12,000 individuals. Most commonly it occurs between the ages of 50-70 years and affects male twice as often as females. A genetic basis has become the single most significance factor and chromosomal aberrations are gaining importance in RCC. Specific chromosomal alterations have been associated with different types. The most common translocation in clear RCC are: t(3;6)(p13;q25); t(3;8)(p14;q24); t(3;12)(q13;q24) t(3;14)(q12;q13). The chromosomes that are involved in deletions include: 3p12; 3p13; 3p14; 3p21; 3p25-26; 6q23-qter; 8p22; 8q11-qter; 9p, 9q; 14q22-qter and 17p13. Chromosome 5 band q22 was found to be duplicated. The chromosomes that are frequently lost include 8, 9, 10, 13 and 14 while chromosomes 3, 12 and 20 are most frequently gained. In papillary RCC, t(X;1)(p11;q21) was involved in translocation, band 17q21 was duplicated the Y-chromosome was lost and chromosomes 7, 12, 16, 17 and 20 were found to be in gain. The frequent loss of chromosomes 1, 2, 6, 10, 13, 17 and 21 were noted in chromophobe tumors. In renal oncocytoma, loss of heterozygosity at on chromosomes 1,2, and 3p has clinical significance. In collecting duct carcinoma, monosomies of chromosomes 1, 6, 14, 15, and 22 and trisomies of chromosomes 7, 12, 16, 17 and 20 have been observed. Loss of 8p and 13q have poor prognosis in collecting duct carcinoma. In addition we present a detailed account of molecular markers and discuss their clinical relevance.

Advances in molecular biology of BCR-ABL gene. *P. Chandra, R.S. Verma.* Department of Medicine, Wyckoff Heights Medical Center, Brooklyn-New York/Weill Medical College of Cornell University, New York, N.Y.

The landmark discovery of the Ph-chromosome in chronic myelogenous leukemia [CML] resulting in a balanced translocation between the long arms of chromosomes 9 and 22 has provided an inspiration for the investigation into the molecular basis of other neoplasia. The hallmark of CML is the formation of a BCR-ABL fusion gene that was thought to play a central role in pathogenesis of CML has now shown a remarkable diverse function in numerous groups of hematologic malignancies. It is the position of the translocation breakpoints in the BCR gene that alters the amount of protein produced after fusion. Certain breakpoints within the BCR gene appear to influence the phenotype of associated leukemias. The p190, p210, and p230 fusion proteins are associated with acute lymphoblastic leukemia [ALL], CML and chronic neutrophilic leukemia [CNL] respectively. The numerous variations of this molecular aberration have now been investigated. For example, there are cases with Ph-negative chromosome who have had neither a discernable BCR-ABL [bcr-negative] rearrangement nor BCR-ABL message and result in highly variable presentation. Then there are patients with reverse BCR-ABL translocation whose prognosis is still poorly understood. What is the prognosis of patients in whom the BCR-ABL gene is translocated to other chromosomes during variant translocations? Is it the BCR-ABL gene resulting in chimeric protein that initiates the event or different fusion proteins drive the expansion of different hematopoietic population? How does the chimeric protein regulate the intracellular signaling pathway? How do p190, p210, and p230 fusion proteins regulate the myeloid cells? A brief account of the latest advances in molecular biology of BCR-ABL gene shall be the subject of debate and discussion.

Identification of New Chromosomal Aberrations as Prognostic Indicators for Pediatric Acute Lymphoblastic

Leukemia. *S.M Chenevert¹, D. Johnston¹, D. Mahoney², F. Elder¹, H. Northrup¹, L. Cooley¹.* 1) Pediatrics, University of Texas, Houston, US; 2) Texas Children's Hospital, TX.

Cytogenetic abnormalities, structural and numerical, have been determined to be significant independent prognostic indicators above clinical features present at diagnosis in pediatric acute lymphoblastic leukemia (ALL) cases. Because long-term survivors of ALL are at risk for late effects of therapy, it is important to identify factors that classify the disease as accurately as possible. Based on the premise that some of the most significant prognostic indicators are seen in only 1-2% of ALL cases, we predicted that there are non-random chromosomal aberrations that are yet to be identified. If a chromosomal aberration is shown to correlate with a very favorable or unfavorable outcome, children with this aberration may be moved from the intermediate-risk group to either low-risk or high-risk therapy group for optimal treatment and outcome. POG provided karyotype, clinical and outcome information on 2,263 pediatric patients diagnosed with ALL and followed over a period of 4.5-13 yrs. The data was analyzed for the identification for new non-random, recurrent chromosomal abnormalities and breakpoints. Ninety-one karyotypic abnormalities were selected. A binary database was set up in order to perform two standard statistical analysis techniques, univariate survival analysis and Cox model regression, that identify chromosome abnormalities that can alter the risk of failure as defined by incidence of relapse. Each abnormality was assessed for overall value as a prognostic indicator as well as its value as a prognostic indicator within the context of clinical parameters and ploidy risk groups. The results of this analysis identified several new non-random recurrent cytogenetic abnormalities that were determined to be significant prognostic indicators. Stratification of each abnormality into several risk groups provided information necessary to propose an algorithm to predict risk of relapse. This model could be used to help more accurately determine the appropriate therapy for patients with ALL, thus improving the quality of life for the patients both during and after treatment.

Cytogenetic abnormalities in leukemic patients from Saudi Arabia: a report of 236 cases. *R.K. Graham, S. Parker, B. Al Muzaini, S. Tashkandi, I. Al Abdulkarym.* Riyadh Armed Forces Hospital Riyadh, Saudi Arabia.

Cytogenetics forms a crucial part in the diagnosis of hematological malignancies due to the presence of common recurring chromosomal aberrations. This study summarizes the cytogenetic findings in 236 leukemic patients with karyotypic abnormalities diagnosed at the RAF Hospital from 1990 to 2000. Cytogenetic analysis was performed on bone marrow samples after 24h incubation using G-banded chromosomes. The objective was to determine the presence of nonrandom and specific chromosomal abnormalities for the Saudi population and make a comparison with previously published hematological cytogenetic aberrations. The report focuses on ALL (Acute Lymphoblastic leukemia), AML (Acute Myeloblastic Leukemia), CGL (Chronic Granulocytic Leukemia), CML (Chronic Myeloid Leukemia) and MDS (Myelodysplastic Syndrome). Overall, 60% were male and 40% female. Clonal evolution was seen in 16% of cases. In ALL, of which there were 48 cases, 27% were adult males and 22% pediatric males in contrast to 4% adult females and 46% pediatric females. The main findings were +4, +6, +8,+10,+12,+21,+X, -7 and -9. Predominant translocations included t(15;17) and t(9;22). In addition, del(1q) and del(6q) were noticeable. In AML (n=54) the majority were adults and the main findings were +4, +8, -7, -14, -20, -X, -Y, 8q-, der(7) and inv(16). Concerning translocations, t(15;17) and t(8;21) were common. Frequent translocations involving chromosome 1 were also noted. In both ALL and AML marker chromosomes were frequently observed. Regarding CGL (n=42) and CML (n=41) the majority of cases were adult and contained the t(9;22). However in CGL, t(9;22) was seen as the sole anomaly in 70% of the cases whereas in CML a significant number also contained additional abnormalities such as +8,+19,+Y, -13 and inv(9). In MDS (n=14) noticeable changes included +8, der(5), -7, and -13. In conclusion, although common recurring chromosomal aberrations are present some variations to the published literature exist. These findings emphasize the importance of classifying the cytogenetic abnormalities in leukemogenesis for this population, which is essential for appropriate clinical care and management.

Racial differences and cytogenetic abnormalities in hematologic malignancies: A five year retrospective study. *L. Cheng, K.H. Ramesh, E.R. Burns, J.C. Park, L.A. Cannizzaro.* Department of Pathology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY.

We studied the cytogenetic makeup of 1100 patients with clinically proven or suspected hematologic malignancies in an ethnically diverse population of New York City. Varying degrees of chromosome abnormalities were detected in 302 patients (238 adults and 64 children). The influence of genetic factors associated with the occurrence and progression of the disease was also investigated. Our cohort was 14% black, 26% Hispanic, 21% white, and 39% others/unknown ethnic origin. Our study concentrated on the cytogenetic classifications, the incidence and types of chromosomal alterations, and the frequency of unrelated abnormal clones among black, Hispanic, and white groups. Our findings showed: 1) Among adult acute myeloid leukemia (AML) patients with chromosome abnormalities involving either 5, 7, or 8, 100% of blacks had involvement vs. 36% for Hispanics ($p=0.011$) and 40% for white ($p=0.016$); 2) Among pediatric acute lymphoblastic leukemia (ALL) patients, blacks had 44% of ploidy anomalies vs. 80% for Hispanics ($p=0.045$); 3) Overall, 12% of Blacks had unrelated abnormal clones vs. 2.5% for Hispanics ($p=0.039$) and 5% for whites ($p=0.085$); 4) There was no association between karyotypic patterns and patients with myelodysplastic syndromes (MDS), lymphoma or multiple myeloma (MM) in the three ethnic groups studied; 5) Overall, there was no statistical difference in the percentage of complex chromosomal abnormalities among these three groups. Our findings suggest an association between ethnic background and chromosomal abnormalities in certain hematologic malignancies. Further studies are needed to determine the genetic mechanisms that may have an influence on hematologic malignancies.

A rare retroperitoneal synovial sarcoma not diagnosed until detection of translocation (X;18)(p11.2;q11.2) and SYT/SSX fusion gene. *Y.C. Dai, H.M. Wu, C.W. Chang, C.C. Tzeng.* Department of Pathology, National Cheng-Kung University Hospital, Tainan, Taiwan, Republic of China.

Malignant spindle cell neoplasms are often a diagnostic challenge in histopathology, regardless of their location. In this report we described a huge primary retroperitoneal malignant spindle cell tumor, measuring 30 x 20 x 15 cm, in a 19-year-old woman. It was not accurately diagnosed until detection of the characteristic chromosomal translocation (X;18)(p11.2;q11.2). Priority of differential diagnoses of retroperitoneal tumors usually depends on patients' age, histopathological and immunological features, their relationship to kidney, and relative incidence at this location. Initially, the tumor of this report was considered to be a Wilms' tumor, with almost purely blastemal component and no immature tubular or glomerular structure. In fact, synovial sarcomas occurred commonly in para-articular regions, with less than 30 tumors arising from retroperitoneum reported in the English literature. Whilst, only two of these retroperitoneal tumors were genetically confirmed so far. This report described the third one well documented by detecting characteristic translocation (X;18)(p11.2;q11.2), as well as demonstrating the presence of SYT/SSX, type II, fusion transcripts with reverse transcriptase polymerase chain reaction (RT-PCR) and sequencing. Furthermore, together with the clinicopathological features of this patient, we supported that the SYT/SSX, type II, fusion genes has a close relationship with monophasic subtype of the synovial sarcoma, as well as a favorable prognosis.

Karyotypic study of two immature ovarian teratomas after chemotherapy induced maturation. *S.A. Faruqi, U. Chapa, R.B. Deger, J.S. Noumoff.* OB/GYN, Div Gynecologic Oncol, Crozer-Chester Medical Ctr, Upland, PA.

We report the karyotypic study of two cases of grade three immature ovarian teratomas after their chemotherapeutic maturation. In the first case the tissue was that of the metastatic mature post-chemotherapy tumor, while in the second case both the primary tumor as well as the residual mature post-chemotherapy tumor were available. The treatment included cisplatin, bleomycin and etoposide combination chemotherapy. Histologically, both the primary tumors contained immature glial tissue but the residual tumors following chemotherapy contained only mature elements. In the first case, the metastasis was found to have monosomies in eleven chromosomes and trisomies in four chromosomes. In the second case, trisomies of chromosomes 7 and 9 were the only two abnormalities which were present both before and after chemotherapy. One other chromosomal anomaly of partial trisomy of chromosomal 11 although present in the primary tumor, was not found in the residual material. It is interesting to note that the MDR-1 gene has been mapped on chromosome 7. Overexpression of its gene product, p-glycoprotein, has been implicated in chemotherapy treatment and worse prognosis in myeloid leukemias. The most important finding in this study is the disappearance of the clone with partial trisomy 11, the chromosome which has the protooncogene cyclin-D, while the other clones survived this particular chemotherapy.

Fine mapping of chromosome 3q amplifications in head and neck squamous cell carcinoma by molecular cytogenetics, and high resolution CGH micro arrays. *S.P. du Manoir¹, K. Wanherdrick¹, D. Muller², J. Abecassis², R. Redon¹.* 1) Human genetics, IGBMC, INSERM-CNRS-ULP, Strasbourg; 2) Paul Strauss center, Strasbourg.

Gain or amplification of the telomeric part of the chromosome 3 is common in HNSCC carcinoma. We analysed a series of 21 well differentiated, node and metastasis negative HNSCC tumors by comparative genomic hybridization. Recurrent gain of the 3q26-qter region was found in 78 % of the cases. Correlation with overall survival suggests a possible predictive value for the 3q26 region gain. After six years, only 50% of patients are still alive in the population with a gain at 3q26 against 90% for the population normal for this locus : patients with 3q26 gain have a shorter survival, even if this difference is not significant (Mantel-Cox test). We extended our series to 45 cases using FISH on interphasic nuclei with a BAC clone containing the PIK3CA gene. In these larger series, after 6 years, 44% of patients with the gain are alive Vs 67% in the normal population : these results suggest a predictive value for 3q26 gain, but were not statistically significant (Mantel-Cox test). Gains targeting 3q26-qter were mapped by interphasic FISH using BAC and YAC clones localized on the long arm of the chromosome 3. Among the tumors exhibiting a gain, five showed a restricted amplification, among them three are amplified only for a part of this region : two different consensus sites of amplification were found around 3q26 and 3q27-qter. Finally, to systematically assess the extend of gains, we designed a high resolution CGH arrays with 60 BAC clones from the chromosome 3 and used it to delineate aberrations in three HNSCC cell lines and the selected tumors with amplification.

Distinct Molecular Cytogenetic Profile in Six Pediatric Patients with Rhabdomyosarcoma. Z. Chen¹, C.M. Coffin², B. Issa¹, S. Arndt¹, R. Shepard¹, L. Brothman¹, J. Stratton¹, A.R. Brothman¹, H. Zhou². 1) Dept Pediatrics/Cytogenetics, Univ Utah Sch Medicine, Salt Lake City, UT; 2) Dept Pathology, Univ Utah Sch Medicine, Salt Lake City, UT.

Rhabdomyosarcoma (RMS) represents the most common soft-tissue sarcoma in children under the age of 15 years. Histologically, RMS can be subdivided into two major subtypes: embryonal (E-RMS) and alveolar (A-RMS) rhabdomyosarcoma, with E-RMS being the most common. Genetically, A-RMS is characterized by a t(2;13) (PAX3-FKHR fusion product) or a t(1;13) (PAX7-FKHR fusion product), whereas E-RMS by gains of chromosomes 2,8,11,12, and 13. In addition, loss of heterozygosity (LOH) at 11p,16q, 6p and 18p has been frequently observed in both types as well. However, molecular cytogenetic studies on other subtypes of RMS are scarce. In the present study we performed cytogenetics, fluorescence in situ hybridization (FISH), Spectral Karyotyping (SKY), and immunohistochemical antibody analysis on six pediatric patients with RMS, including one A-RMS, three E-RMS, one botryoid RMS, and one anaplastic non-classified RMS (N-RMS). Our findings in A-RMS and E-RMS were generally consistent with previous reports; however, i(15)(q10), del(4)(q21q31), and del(9)(p21) observed here have been rarely reported in the literature. Importantly, our botryoid RMS showed a cytogenetic profile consistent with E-RMA, further confirming it is a variant of E-RMA. An add(11)(q21) observed in this tumor, together with a t(8;11)(q12-13;q21) reported previously, highly indicate that 11q21 rearrangements may be specifically related to botryoid RMS. In addition, our N-RMS expressed a cytogenetic pattern similar to that observed in E-RMS, thus providing strong genetic evidence that some anaplastic N-RMS may be a very anaplastic form of E-RMS. Immunohistochemical stains in this tumor also displayed high reactivity for p53, diffuse reactivity for vimentin and desmin, and focal reactivity for muscle specific actin. Anaplasia in RMS is a poor prognostic feature, as is nuclear p53 expression.

Analysis of a homozygous *BRCA1* mutant breast cancer cell line using CGH, SKY and LOH: A model for the comprehensive evaluation of publicly available tumor cell lines. *D. Boles¹, E. Cedrone¹, L. Cavalli², J. Rone², C. Long¹, D. Burkhardt¹, P.S. Donover¹, B. Haddad².* 1) American Type Culture Collection, Manassas, VA; 2) Georgetown University Medical Center, Washington, DC.

A large number of tumor cell lines are available in the public domain. Many of these cell lines have been characterized using conventional cytogenetic studies and nearly all of those examined carry chromosomal aberrations. Such studies, while important, need to be complemented using newer approaches. Today, new molecular technologies including comparative genomic hybridization (CGH), spectral karyotyping (SKY) and loss of heterozygosity (LOH) studies allow tumor genomes to be surveyed for chromosomal aberrations in a more thorough and comprehensive way thus allowing the identification of tumor-specific chromosomal anomalies with a high degree of accuracy. The availability of such information on publicly available tumor cell lines will provide researchers with a valuable resource. As a model of such an approach, we have used CGH, SKY and LOH to characterize the cell line HCC1937 (ATCC # CRL-2336) derived from an identical triplet who developed breast cancer at age 24 and has a homozygous germline mutation in *BRCA1* (*BRCA1* 5382insC). Initial analyses using classical cytogenetics revealed a highly transformed karyotype with a modal chromosome number of 100 and at least 43 marker chromosomes involving nearly every chromosome. The complexity of this karyotype prevented clear breakpoint designations or identification of partner chromosomes in all rearrangements. However, the combination of SKY and CGH allowed a comprehensive characterization of these chromosomal findings. LOH data provided further insights on specific genes lost. With such an approach, description of newly identified aberrations is possible, previously unidentified structural rearrangements are resolved and new breakpoints are defined. We conclude that such studies should be undertaken to complement existing conventional cytogenetic information on publicly available tumor cell lines in a more systematic way.

Transient Leukemia with Trisomy 21 Showing T-Cell Phenotype. *Q.L. Hu, H.O. Shah, I. Akhtar, S. Korsuri, D. Sabatino, J. Sherman, B. Feldman, J. Lin.* Pathology and Laboratory, Nassau County Medical Center, East Meadow, NY.

Nassau County Medical Center, East Meadow, New York, Health Science Center, State University of New York at Stony Brook, New York. Transient leukemia (TL) has been reported in 10% of infants with Down syndrome and showed predominately megakaryoblastic phenotype. TL usually disappears spontaneously. But in approximately 25 percent of the cases who recover will develop acute megakaryocytic leukemia (M7) in next four years. Its clonal proliferation and tissue infiltration favor TL to be a specific form of leukemia. Here we present a case of TL with T-lymphocyte phenotype by cytochemical approach. The full term infant was born to a 20-year old female spontaneous with delivery. Physical examination showed features of Down syndrome with yellow, crusty skin rash on face and hepatosplenomegaly. Peripheral blood cell counts (PBC) at birth: WBC 47.7K/cubic millimeter, blasts 42 percent, RBC 4.53 million/cubic millimeter, and platelets 122.8K/cubic millimeter. Bone marrow aspirate at the second week of birth showed 27.3 percent of blasts, which were positive for acid phosphatase in paranuclear pattern, negative for alpha-naphthyl acetate esterase, ASD-chloroacetate esterase and CD61. Cytogenetic study of the bone marrow confirmed trisomy 21. The patient was managed with supportive therapy and clinical observation alone. On 92nd days of birth, the WBC count was 9.1K/cubic millimeter with a normal differential and there were no blasts present. The baby has since been doing well up to now at nine months of age. Even though majority of TLs is of megakaryocytic nature, TLs of T-lymphocyte phenotype is very rare. TLs may possess totipotent properties to differentiate into other cellular lineages, such as this, into T-cell phenotype.

Hepatoblastoma in a child with mosaic trisomy 18. *B. Kohn¹, K. Ayelew¹, M. Mannikal¹, S.M. Kleyman², M.J. Macera², R.M. Chandra², and R.S. Verma².* 1) Long Island College Hospital, Brooklyn, NY; 2) Wyckoff Heights Medical Center, Brooklyn- New York Hospital/Weill Medical College of Cornell University, New York, NY.

Hepatoblastoma is a rare tumor occurring most frequently in children under three years of age. Adult cases have occasionally been reported. Most of the cytogenetic evaluation has been carried out on tumor-derived cell lines. No consistent hepatoblastoma-specific genetic markers have been established. Most hepatoblastomas are diploid or hyperdiploid, often displaying trisomies for chromosomes 2 and 20, 2q+, 11p-, and 13q-. A 16 month-old female, the product of a 37-week gestation to a 41-year-old mother and 42 year-old father was referred for failure to thrive and severe hypercalcemia. Her birth weight was 3 lbs. The pregnancy was complicated by premature labor. A brief clinical presentation included failure to thrive with poor weight gain and delayed motor and verbal milestones. Clinical examination revealed dysmorphic features including strabismus, prominent occipital and narrow bifrontal diameter, low-set ears, narrow palatal arch, micrognathia, hypoplasia of the nails on fingers and toes, short sternum, small areola and small pelvis. The liver was enlarged to 3 cm below the right costal margin and hypotonia was detected. Neurological development was delayed. Abdominal CT denoted a large heterogeneous mass in the right hepatic lobe. Tumor pathology confirmed a hepatoblastoma. Cytogenetic findings with G-banding revealed a mosaic karyotype for chromosome 18 {i.e. 47,XX,+18[37]/46,XX[13]} in her peripheral blood. Tissue was unsuccessful in culture, however, interphase cytogenetics by FISH revealed the presence of both cell lines, suggesting that both normal cells and tumor cells were present. Since neoplasias arise from a single cell, only one tumor cell line was expected. It has been suggested that trisomy 18 may predispose to both hepatoblastoma and Wilms' tumor. This is a rare tumor and unfortunately, cytogenetic findings are not available, so a definitive conclusion concerning the chromosomal basis of hepatoblastoma could not be drawn.

Identification of two distinct variant *bcr/abl* translocations in Ph-negative Chronic Myeloid Leukemia cases by FISH. M. Mohammed, S. Shin, G.J. Schiller, C.L. Sawyers, P.N. Rao. UCLA School of Medicine, Los Angeles, CA.

Approximately 5-10 % of patients with morphologically typical CML, appear to be Ph-negative, presenting with variant translocations in which the *bcr-abl* fusion gene may be cytogenetically masked. The mechanisms of their formation and the clinical significance are still unclear. The location of the fusion gene on chromosomes other than 22q-remains rare. We present here two CML patients with Ph-negative karyotypes and unusual *bcr-abl* presentations. **Case 1** : A 42-year-old female, with morphologic findings consistent with CML. Bone marrow cytogenetics revealed a Ph-negative abnormal karyotype, 46,XX,t(13;20)(q14;q13.1)c. To confirm the CML status, FISH with *bcr-abl* probe (Vysis) was performed. The fused gene was found to be localized to chromosome 9q34, and not on chromosome 22q11. Additional FISH analyses with the DiGeorge/VCFS probe (Vysis), showed both, the TUPLE1 probe which is slightly distal to the *bcr* probe, and the ARSA (control) probe, hybridized to chromosome 22. Given the close proximity of the TUPLE 1 probe to the *bcr*, it is likely that a discrete interstitial insertion of the *bcr* into the 9q34 as a secondary event, occurred in this patient. **Case 2**: A 47-year-old white male, with CML in blast crisis. The pretreatment karyotype was Ph-negative and abnormal: 46,XY,t(17;19)(q23;q13.1). However, FISH analyses revealed that the *bcr-abl* fusion gene was located on chromosome 22. Uniquely, an *abl* probe signal was observed on both 9q34 homologues, although one of the signals appeared smaller(?*abl* gene split). FISH analyses on a bone marrow sample after treatment, revealed a second distinct clone (33%). As with the initial clone, the *bcr-abl* fusion gene was located on chromosome 22, but unlike the initial clone, the extra *abl* signal was now interstitially located at 9q22 region, instead of 9q34 (?inversion). The exact mechanism of such a novel presentation of the fusion gene remains unclear. Further FISH and molecular studies to unravel some of these mechanisms are in progress. These cases highlight the heterogeneity with which the *bcr-abl* fusion gene may be formed and the usefulness of multicolor metaphase FISH in detecting variant *bcr-abl* translocations.

Defects in Methylthioadenosine Phosphorylase are Associated with but not Responsible for Methionine-Dependent Tumor Cell Growth. *B. Tang, Y.N. Li, W.D. Kruger.* Division of Population Science, Fox Chase Cancer Center, Philadelphia, PA.

A large proportion of human tumor derived cell lines and primary tumor cells show methionine-dependent growth. This phenomenon refers to the ability of cells to grow in media containing methionine but the inability to grow on media supplemented with methionines precursor, homocysteine. Methionine can be formed by two different pathways; the recycling pathway and the salvage pathway. To discover the basis for methionine-dependent growth we have analyzed twelve tumor cell lines and two non-tumor derived cell lines for defects in two key genes in different methionine synthetic pathways. We found little evidence that defects in methionine synthase (MS) expression or mutations in the MS gene are correlated with methionine-dependent growth. However, we did find a correlation between methionine-dependent growth and defects in expression of methylthioadenosine phosphorylase (MTAP), a key enzyme in the salvage pathway. Three of the four cell lines lacking detectable MTAP protein were unable to grow in homocysteine media, while all six of the MTAP positive cell lines tested showed strong growth. However, when we introduced the MTAP cDNA into MTAP deficient MCF-7 cells, the resulting cell line was still defective in growth on homocysteine, although it could now grow on the salvage pathway precursor methylthioadenosine. These findings indicate that salvage pathway defects are not causally related to methionine-dependent growth.

Genomic Alterations in Uterine Leiomyosarcomas: Potential Markers for Clinical Diagnosis and Prognosis. J.

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Genomic alterations were analyzed in 21 uterine leiomyosarcomas (ULMS) by comparative genomic hybridization. DNA copy number changes were detected in all 21 tumors. The most frequent losses were 13q (16/21=76%), 10q (13/21=62%), 16q (8/21=38%), 12p (7/21=33%) and 2p (9/21=43%). The most common gains were 17p (8/21=38%), Xp(7/21=33%) and 1q (7/21=33%). High copy number gains (ratio>1.5) were identified in Xp, 1q and 17p. Loss of 13q was identified in both low grade and high-grade tumors. Inactivation of a tumor suppressor gene in 13q may be an early event in the development of leiomyosarcomas. Loss of 10q, 2p, 12p and gains of 1q as well as 17p were frequently found in high-grade tumors and recurrent tumors. Inactivation of tumor suppressor genes and activation of oncogenes in these regions may be associated with a more aggressive behavior of ULMS. Patients with just loss of 13q and without other alterations listed above had longer survival. Gains of Xp, 17p, 1q and losses of 13q, 10q, 16q, 12p and 2p have been reported in extra-uterine leiomyosarcomas. Our findings indicate that the pathogenesis of uterine leiomyosarcomas and extra-uterine leiomyosarcomas follow the same genetic pathways.

Statistical analysis for rb-1 monoallelic deletion by FISH is crucial in multiple myeloma diagnosis. *G. Lu, R.G. Best.* Obstetrics & Gynecology, University of South Carolina, Columbia, SC.

Multiple myeloma (MM) is a common B-cell malignancy for which activation of oncogene and loss of function of tumor suppressor genes have both been proposed. Of the tumor suppressor genes studied, loss of the retinoblastoma susceptibility gene rb-1 on 13q14 is most frequently reported with an estimated frequency as high as 52%. The loss of rb-1 gene is associated with late stage and poor prognosis of the disease. The rb-1 deletion is usually monoallelic and is often undetectable by conventional cytogenetic technique, thus requiring application of fluorescence in situ hybridization (FISH) in diagnosis. We have recently identified three cases of MM with monoallelic deletions of rb-1 with between 5% and 70% mosaicism. Of the three cases, only one showed conventional G-band deletion of 13q14 with just 2 out of 20 cells exhibiting the deletion. Rb-1 deletion by FISH was observed in only 5% of interphase cells. Rb-1 deletions in MM in the published literature are almost always monoallelic and mosaic, however, the degree of mosaicism in interphase exhibits variability from case to case, and between interphase and metaphase cells. Our findings and those reported in the literature emphasize the importance of using clearly defined statistical methods and protocols in each individual laboratory for discriminating true mosaicism from pseudomosaicism when testing interphase cells for deletions on 13q14. We discuss our protocol and its application in other laboratory settings including the use of sensitivity and specificity of the probes in determination of standards for discriminating true mosaicism from technical artifact, and the lower limits of detection of mosaicism for various interphase cell sample sizes.

Heterogeneity of t(11;17) translocations in leukemia. *S. Strehl¹, M. König¹, O.A. Haas^{1, 2}*. 1) CCRI, Children's Cancer Research Institute, Vienna, Austria; 2) Ludwig-Boltzmann Institute for Cytogenetic Diagnosis (LBICD).

Rearrangements of the MLL gene on chromosome 11q23 account for 5-10% of acquired chromosomal aberrations in children and adults with acute lymphoblastic and myeloblastic leukemia, poorly differentiated or biphenotypic leukemias and myelodysplastic syndromes. Strikingly, involvement of at least 40 different chromosome regions in 11q23 translocations has been determined. Twenty-three fusion partner genes have been cloned and partially characterized to date. Two translocations occur at 17q21 and 17q25 each accounting for 1-1.5% of all MLL rearrangements. AF17 localized at 17q21 and MSF at 17q25 have been cloned as fusion partners of MLL. In acute promyelocytic leukemia also a variant of the more common t(15;17)(q22;q21) resulting in the chimeric PML/RARa fusion gene has been observed. In the t(11;17)(q23;q21) variant the promyelocytic leukemia zinc finger (PLZF) gene (11q23) is fused to RARa (17q21). Recently, based on FISH data also a putative fusion between MLL and RARa was suggested and still another MLL fusion partner, GAS7, is localized at 17p13. We have identified seven patients with acute leukemia carrying a t(11;17)(q23;q21-25) by cytogenetics and/or whole chromosome painting and in six of them rearrangement of the MLL gene was detected by FISH analysis using a YAC clone that spans the MLL breakpoint region. RT-PCR for the detection of the MLL/AF17 and the PLZF/RARa fusion genes was performed and surprisingly only one case was positive for MLL/AF17 suggesting involvement of other MLL partner genes. However, also RT-PCR for the MLL/MSF fusion could not detect the particular chimeric transcript at least in three of the samples. In order to further characterize the chromosomal breakpoints FISH with an MLL YAC clone and a RARa probe was performed and three different hybridization patterns were detected: (i) MLL splits and RARa remains on the der(17), (ii) MLL splits and RARa is translocated to the der(11), and (iii) MLL is translocated to the der(17) and RARa splits. These investigations will contribute to the accurate classification of distinct t(11;17) translocations and possibly lead to the detection and cloning of new MLL fusion partners.

Gene amplification occurs via breakage-fusion-bridge cycles in oral cancer cells. X. Huang¹, M. Shuster^{1,2}, T.E. Godfrey^{1,3,4}, W.S. Saunders^{2,4}, B.M. Gharaibeh¹, S.M. Gollin^{1,4}. 1) Department of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; 2) Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA; 3) Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA; 4) University of Pittsburgh Cancer Institute, Pittsburgh, PA.

Chromosomal instability is an important feature of cancer cells. Gene amplification is a common genetic alteration in tumors and is one mechanism for producing a higher level of gene product. Oral squamous cell carcinomas (OSCC) are characterized by complex, often near-triploid karyotypes with structural and numerical variations on a theme of clonal chromosomal alterations. About half of OSCC and derived cell lines express a homogeneously staining region (hsr) comprised of amplified chromosomal band 11q13. We and others have proposed that gene amplification occurs via breakage-fusion-bridge (BFB) cycles. We used FISH with probes to D11Z1, *RINI*, and cyclin D1 (*CCND1*) to examine the amplicon in our oral cancer cell lines and observed a predictable pattern of organization, an "amplification fingerprint" consistent with BFB cycles. This is characterized by an inverted duplication chromosome pattern in the region of amplification, with genes from the segment just distal to the proximal chromosomal breakpoint duplicated and flanking both sides of the amplified genes in the amplicon. In addition, FISH analysis confirmed the hypothesis by revealing the presence of the amplified *CCND1* gene in the anaphase bridges between nascent oral cancer cells. To accurately map the proximal and distal breakpoints involved in the BFB cycles, we have used quantitative microsatellite analysis (QuMA). Of the 19 OSCC cell lines with 11q13 amplification examined to date, our data show that the proximal and distal breakpoints are the same in 63% and 53%, respectively. This may indicate the presence of one or two as yet unidentified breakage hotspots or fragile sites that are responsible for 11q13 amplification by BFB cycles. These results provide the first cytological evidence that gene amplification in oral cancer cells occurs via breakage-fusion-bridge cycles.

A highly specific and sensitive fluorescence in situ hybridization (FISH) assay for the detection of t(4;11)(q21;q23) in acute leukemias. *O.A. Haas¹, S. Strehl¹, M. König¹, M. Reichel², R. Marschalek².* 1) Children's Cancer Research Institute (CCRI) and Ludwig-Boltzmann Institute for Cytogenetic Diagnosis (LBICD), St Anna Children's Hosp, Vienna, Austria; 2) Department of Genetics, University of Erlangen, Germany.

The t(4;11)(q21;q23) is one of the most common 11q23 abnormalities. It occurs typically in infant leukemias and topoisomerase II inhibitor induced secondary acute leukemias. The t(4;11) fuses the *AF4* and the *MLL* genes and generates a chimeric *MLL/AF4* mRNA that is easily detectable with RT-PCR. In contrast, 11q23 abnormalities can only be detected indirectly with FISH by hybridization of clones that span or flank the *MLL* gene. Until recently, two YAC clones (13HH4 and yB22B2) were used for this purpose. They are split by translocations involving the *MLL* gene. Unfortunately, these YAC clones do not identify those approximately 20% of cases in which the 3'-*MLL* part is deleted. Therefore, new two color *MLL* probes were developed that result in the segregation of normally overlapping green- and red-labeled clones in case of translocations. Whereas such assays reliably detect all the translocations that involve *MLL*, they provide no information about the translocation partner in interphase. We therefore designed a FISH assay for the specific and sensitive detection of the t(4;11)(q23;q21) that can be applied for both interphase and metaphase analysis. Our differentially labeled *MLL* YAC (13HH4) and *AF4* PAC clones are both split in case of a t(4;11). Therefore, FISH generates two red and two green signals in normal cells, whereas in case both the reciprocal gene fusions are present in t(4;11)-positive cells, one red and one green signal occur together with two green/red co-localized signals. We evaluated the specificity and sensitivity of this FISH assay on normal controls as well as on serial dilutions of a t(4;11)-positive leukemia cell line in normal cells. To date, we have successfully studied 18 *MLL/AF4*-positive cases with varying amounts of t(4;11)-positive cells as well as unusual cases that had the der(4) chromosome either deleted or duplicated.

Camptothecin-induced DNA damage identifies a deficiency in a double strand break repair subpathway in Ataxia-Telangiectasia which promotes multiple chromosome exchanges in probands and obligate heterozygotes.

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A-T cells are chromosomally unstable, and are hypersensitive to ionizing radiation and other agents causing DNA double strand breaks (DSBs). Chromosome studies have suggested a defect in DSB repair, but direct measurements are less clear, and the underlying defect and basis for cell killing are uncertain. Here we use the topoisomerase I inhibitor camptothecin (CPT), which induces DSBs predominantly in replication forks, and show that A-T cells are defective in the repair of this subclass of DSBs. After CPT treatment, A-T cells pass to G2, where they remain; normal cells repair most of their DSBs and arrest briefly in G2. The frequency of CPT-induced chromosome aberrations (viewed in prematurely condensed G2 chromosomes) in each of a wide variety of A-T cells examined, including different ATM gene mutations, is abnormally high; aberrations are S-phase-derived and many are multiple illegitimate chromatid exchanges. In normal cells the aberrations are mostly chromatid breaks. Obligate A-T heterozygotes also show more aberrations than normal controls. FISH analysis using whole chromosome paints reveals the complexity and type of exchange aberrations. The data suggest that ATM, the A-T protein, recognises DSBs in active replicons and helps to suppress illegitimate S-phase recombination.

The Role of RxFISH in the Delineation of Complex Chromosomal Abnormalities in Hematologic Disorders. *J.H. Hersh¹, M.J. Barch¹, D.A. Stevens², F.F. Yen¹, E. Jusufbegovic¹, B. Weisskopf¹.* 1) Dept Pediatrics/CEC, Univ Louisville, Louisville, KY; 2) Nortons Hospital, Louisville, KY.

Complex karyotypes frequently are found in hematologic disorders, which are difficult to characterize by conventional banding. The development of fluorescence in situ hybridization (FISH) represented an important step in overcoming this problem, but has been limited by its inability to detect the entire genome in a single application. Cross-species color banding (RxFISH) is a new FISH technique utilizing probes from flow-sorted, differentially labeled gibbon chromosomes which overcomes this problem by examining the entire karyotype at one time. Harrison et al, Teixeira et al and Micci et al demonstrated the usefulness of RxFISH to characterize chromosomal rearrangements in patients with leukemia and solid tumors incompletely identified by G-banding. We present data comparing results of conventional banding and RxFISH on bone marrow specimens performed on 50 patients with hematologic disorders. In 14/50 (28%), a chromosomal abnormality was detected by both methods. Diagnostic entities included 8 patients with leukemia, 3 with lymphoma, 2 with thrombocytopenia and 1 with myelodysplastic syndrome. RxFISH confirmed a balanced translocation in 4 patients and deletion of a #20 chromosome and presence of a small marker chromosome in two others that had been identified by G-banding; however the origin of the marker could not be determined by either technique. In the other 8 patients, complex karyotypes were recognized on conventional banding, although complete characterization could not be achieved. RxFISH provided additional information about these abnormalities, which were confirmed by FISH studies. Our results support the previous conclusion that RxFISH provides more accurate information about complex chromosome abnormalities present in hematologic disorders and can serve as a valuable adjunct to conventional banding in the interpretation of complex karyotypes in these conditions.

Design of an Automated Spot Counting Program for Interphase FISH on the Tissue Microarray. G.H.

HOSTETTER¹, C.L. ANDERSEN¹, L. BUBENDORF¹, J. KONONEN¹, E. DOUGHERTY², A. GRIGORYAN², O. KALLIONIEMI¹. 1) Cancer Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Computer-Assisted Medical Diagnostic Imaging (CAMDI) Laboratory, Texas A&M University, College Station, TX.

Tissue microarray technology (Kononen et al. 1998) facilitates high-throughput analysis of genetic changes in cancer and other diseases. Up to 1000 tissue specimens can be placed on a single microscope slide and subjected to molecular analyses, such as FISH. Consecutive tissue microarray sections can be analyzed with probes or antibodies to perform comprehensive molecular profiling of the tissues. In the past two years, our laboratories have performed close to 20,000 FISH experiments. The most time-consuming part is the manual scoring of the FISH signals. Here, we report the development of an automated system that acquires 3-dimensional images of tissue sections, and automatically analyzes the relative copy number of target loci. A stack of 12 consecutive confocal images are acquired with a Quantix CCD camera attached to a Axioplan2 microscope equipped with a confocal CARV module. The FISH spot counting algorithm defines the signals in three dimensions, allowing separation of closely clustered signals. The program was tested on 17 images from breast cancers hybridized with HER-2 and chr 17 cen probes (Vysis Inc.). Detection of HER-2 amplification showed 94% agreement between manual and automated scoring and the correlation between the signal counts was $r=0.468$ ($p<0.001$). We then designed three adaptations: 1) Improved definition of the lower limit of size and intensity for a real FISH spot, 2) Improved analysis of tight clusters, and 3) User interaction by gating out regions of autofluorescence. In summary, development of the tissue microarray technology and automated FISH analysis have now paved the way for a high-throughput automated analysis of genetic alterations in thousands of tissues. We are now carrying out a clinical study to test the performance of the system in a large sample set of breast cancers hybridized with the HER-2 probe.

Comparative analysis of dual versus single color *MLL* probes: Early relapse in t(4;11) leukemia detected by dual color probe only. J.L. Murata-Collins, P. Gaytan, G. Alvarez, M.L. Slovak. Dept Cytogenetics, City of Hope National Med Ctr, Duarte, CA.

Accurate detection of disruptions involving the *MLL* locus, mapped to chromosome band 11q23, is critical in cancer cytogenetics, providing information necessary for both disease classification and ascertainment of prognostic risk category. After initial disease assessment and treatment, monitoring by fluorescence *in situ* hybridization (FISH) to detect minimal residual disease or early relapse is key to successful clinical intervention. Previous in-house validation studies for a single color *MLL* probe established a value <10%, for translocations, to be within background limits selected to maximize specificity [sensitivity, 91.7%, with 95% confidence intervals (CI) of 61.5%-99.8%; and specificity, 97.8%, with 95% CI of 88.2%-99.9%]. To determine whether a dual color *MLL* probe would increase sensitivity of detection, a case comparison study was performed, matching both types of *MLL* FISH probes and classic cytogenetics (CG) for 19 abnormal specimens, including translocations and deletions, and 11 normal controls. In 17 specimens, an 11q23 translocation was detected by CG in 5% - 100% of cells; eight with t(4;11), three with t(11;19), three with t(6;11), and two with t(9;11). Using a single color *MLL* probe, three t(4;11) specimens gave false negative (FN) results, under the 10% background limit. Using a dual color *MLL* probe, all translocation specimens scored positive, ranging from 10%-100%, with controls scoring between 0%-2% false positive. The three FN studies by single color probe, 0.6%, 1.4%, and 8%, scored 14.5%, 15%, and 23.5% positive, respectively, by dual color probe. In two 11q23 deletion cases, both probes gave normal signal patterns, indicating neither deletion encompassed the *MLL* locus. Despite an increased potential for false positives, due to the appearance of split signals in interphase nuclei, the dual color *MLL* probe increased both sensitivity and specificity in detecting *MLL* translocations, reducing background limits to £3% [sensitivity, 100.0%, with 95% CI of 80.5%-100.0%; and specificity, 100.0%, with 95% CI of 71.5%-100.0%].

Duplicated 20q deletion and acute myelomonocytic leukemia (M4). *S.L. Wenger, D. Wonsettler, W.W.L. Chang.*
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A 59-year-old hypertensive white male was admitted to the hospital with shortness of breath, tightness in chest and weakness. He was found to have a pericardial effusion and underwent pericardiocentesis with placement of a drainage catheter, which continuously drained bloody fluid. He had marked leukocytosis and thrombocytopenia since surgery for a ruptured appendix 7 months ago. His leukocyte count was up to 140,000 with left shift and with 67% of atypical monocytoïd cells; the platelets were down to 23,000. Flow cytometry and smear of bone marrow aspirate were consistent with AML M4. A bone marrow aspirate showed a karyotype of 46,XY,del(20)(q11.2q13.3)[12]/47,XY,del(20)(q11.2q13.3), +del(20)(q11.2q13.3)[8]. The majority of cases with 20q deletion are associated with myeloid disorders, however, duplication of the 20q deletion has rarely been reported. The patient's hemorrhagic diathesis and cardiac tamponade aggravated and he expired 7 days after admission. Autopsy confirmed the clinical findings of AML M4. Marked cardiomegaly and extensive fibrinous pericarditis were evident. Hepatosplenomegaly was also present. Many foamy macrophages with bubbling cytoplasm in the spleen, liver, bone marrow and lymph nodes were suggestive of Niemann-Pick disease, type E. AML has not previously been reported with Niemann-Pick disease.

Pentasomy 8 in pediatric myelodysplastic syndrome. *S. Wu*¹, *W. Wong*². 1) Division of Medical Genetics, Dept Pediatrics, Children's Hosp, Los Angeles, Los Angeles, CA; 2) Division of Hematology, Dept Pediatrics, Children's Hosp, Los Angeles.

Myelodysplastic syndrome (MDS) is a disease affecting mainly the elderly and it is rare in children. We present a case of pediatric MDS with a unique numerical chromosomal anomaly pentasomy 8. To our knowledge, this is the first reported pediatric MDS with pentasomy 8. The patient is a 2 year-old Hispanic male who presented with lethargy, vomiting, abdominal pain and swelling, tactile fever and a diffuse rash. Bone marrow aspirate and biopsy revealed dyserythropoiesis with marked erythroid hyperplasia, moderate megaloblastic changes and binucleated erythroid cells, irregular nuclear contours and cytoplasmic bridging. There was a marked decrease in granulocytes with 4.5% blasts and rare megakaryocytes. Karyotypic analysis showed a major clone with three additional chromosome 8s, one additional 19 and 21 with a minor clone showing an apparently normal male karyotype. The karyotype for the bone marrow cells at diagnosis was 51, XY, +8, +8, +8, +19, +21 [16]/ 46,XY [4]. FISH test using centromeric probe of Chromosome 8 demonstrated a wide range of copy number of chromosome 8 in the bone marrow cells with 0.5 % of monosomy, 39 % of disomy, 7.4 % of trisomy, 14.4 % of tetrasomy, 38.2 % of pentasomy and 0.5 % of hexasomy cells. The coexistence of pentasomy 8, tetrasomy 8 and trisomy 8 in his marrow cells suggests that the pentasomy cell line may be preceded by a tetrasomic or trisomic cell line or both, which went undetected by metaphase cytogenetics.

Karyotype refinement in five patients with Acute Myeloid Leukemia using spectral karyotyping. *G. Palka^{1,2}, D. Fantasia^{1,2}, P. Guanciali Franchi^{1,2}, A. Spadano³, E. Morizio^{1,2}, F. Capodiferro^{1,2}, R. Mingarelli⁴, P. Di Bartolomeo⁵, L. Stuppia¹, G. Calabrese^{1,2}.* 1) Dept Scienze Biomediche, Univ G D'Annunzio, Chieti Scalo, Chieti, Italy; 2) Servizio di Genetica Umana, Ospedale Civile, Pescara, Italy; 3) Dipartimento di Ematologia, Ospedale Civile, Pescara, Italy; 4) CSS-IRCCS Mendel, Roma, Italy; 5) Unit di Terapia Intensiva Ematologica per il trapianto ematopoietico, Ospedale Civile di Pescara, Italy.

Cytogenetic analysis combined with different banding techniques allows to identify many chromosome rearrangements specifically associated with hematological disorders. However, poorly spreaded metaphases, chromosome fuzziness, highly rearranged karyotypes with numerous indecipherable marker chromosomes are often found in leukemia. These abnormalities are usually difficult to define using standard techniques and FISH. Recently a new technique named SKY provided additional information resulting into the precise identification of chromosome markers, complex chromosome rearrangements, and cryptic translocations. We describe 5 patients with acute myeloid leukaemia (AML) referred to the Hematology Department of Pescara Hospital. According to FAB classification, one patient, a 55 year old man, had AML-M0; the second and the third patients, two female 62 and 75 year old, respectively, had AML-M1; the fourth, a 74 year old woman, suffered of an AML-M2, and the last patient, a 34 year old woman, had AML-M4. In these 5 patients cytogenetic investigation using G-banding technique showed the presence of several complex rearrangements and indecipherable markers. For this reason SKY analysis was performed. This analysis allowed the precise definition of the karyotype in all patients by identifying 4 chromosome markers, 12 translocations and 2 insertions misidentified or undetected by standard banding technique. FISH analysis with painting and specific probes confirmed SKY results in all cases. Although at present SKY analysis has a major limitation being unable to identify chromosome breakpoints, it is of great usefulness for classifying complex rearrangements and for detecting the origin of the disease. This is a crucial point for diagnosis and prognosis assessment.

Der(Y)t(Y;1)(q12;q12) associated with myelodysplasia: Case report and review of the literature. *M.H. Abdel-Rahman, C.E. Glasure, M.A. Caligiuri, K.S. Theil.* Department of Pathology, Divisions of Cytogenetics and Laboratory Hematology; Department of Internal Medicine, Division of Hematology-Oncology, The Ohio State University, Columbus, OH.

Cytogenetic analysis of a bone marrow aspirate from a patient with a long standing history of refractory anemia revealed a clonal 46,X,der(Y)t(Y;1)(q12;q12) karyotype. The hematological examination and bone marrow aspirate morphology were not conclusive for a diagnosis of MDS, and the presence of a clonal chromosomal abnormality was a key diagnostic feature. This abnormality has been reported previously in eleven patients, and is highly associated with myelodysplastic syndromes. The chromosomal breakpoints were confirmed to be in satellite III DNA (DYZ1) of the Y chromosome and in the satellite II/III DNA in chromosome 1 by fluorescence in situ hybridization. A literature review of the reported cases with der(Y)t(Y;1) is presented.

Five cases with segmental amplification of 11q23 region identified by FISH in patients with AML, sAML or HIV related lymphoma and a poor prognosis. *L. Parsons¹, L. Mak¹, P. Dighe², T. Saphner³, M.K. Crow⁴, M. Scott⁵, K.S. Reddy¹.* 1) Dept Cytogenetics, Quest Diagnostics Inc., San Juan Capist, CA; 2) Stockton Hematology Oncology Medical Group, Stockton, CA; 3) Green Bay Oncology Ltd., Wisconsin; 4) Diagnostic Clinic of Houston, TX; 5) Indian River Memorial Hospital, Vero Beach, FL .

Five cases with segmental amplifications of 11q23 region were detected by FISH. The amplification was either contiguous amplification on chromosome 11, a hsr or multiple markers of 11q23 region. These markers were derivative chromosomes, or isochromosomes involving 11q23 region. Amplification of 11q23 region was associated with a complex karyotype in all cases. 4 cases were myeloid leukemias belonging to either AML-M5a or AML-1 subtype and were resistant to treatment and the case with a hsr was an AIDS associated lymphoma. Conventional cytogenetic analysis and fluorescence in situ hybridization (FISH) studies using MLL,11 painting, or 11 centromere probes were necessary to ascertain the segmental amplification of the 11q23 region. Amplification of the MLL gene or other genes that map to 11q23qter may be responsible for the poor prognosis in these cases.

Detection of homogeneous staining region (hsr) in acute lymphoblastic leukemia (ALL). *J. Xu¹, F. Ling², B. Dingle².* 1) Pathology & Molecular Medicine, McMaster Univ, Hamilton, ON., Canada; 2) Grand River Hospital, ON., Canada.

Hsr and double minutes (dmin) are cytogenetic evidence of gene amplification and occur frequently in solid tumors, such as brain, breast and ovarian carcinomas. Detection of gene amplification in solid tumors generally predicts a poor prognosis and demands more aggressive treatment. Hsr and dmin are rare findings in hemotologic malignancies and have been reported in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). We report a case of the presence of hsr in ALL. The patient was a 79-year-old woman who was admitted to the hospital for investigation of increasing fatigue and anemia. Her only significant past medical history was idiopathic thrombocytopenic purpura about 20 years ago. Her blood counts were as follows: Hb 101 g/L, WBC $19 \times 10^9/L$ and platelet $8 \times 10^9/L$. The peripheral blood smear showed leucocytosis with 50% blasts. The bone marrow examination showed hypercellular particles with 80% blasts, confirming the diagnosis of acute leukemia. The blasts were negative for myeloperoxidase and flow cytometry showed immunophenotypic features characteristic of B-precursor lymphoblasts (TdT+, CD19+ and CD34+). The overall findings were consistent with ALL, null phenotype, L2 type under the FAB classification. The patient developed an infection and died shortly after admission. Analysis of 24 GTG banded metaphases prepared from the patient's bone marrow showed multiple numerical and structural chromosome abnormalities. The karyotype was 41~45,XX,-3[24],-5[9],del(5)(q15q34)[15],add(6)(p11.1)[24],hsr(11)(q21)[21],der(13:21)(q10:q10)[22],-14[5],inv(16)(p13q22)[15],-16[4],add(17)(p11.2)[24],-20[23],-22[19],+mar1[24],+mar2[10],+mar3[6],+mar4[3][cp24]. Among these aberrations, monosomy 22 is the only one as a common primary anomaly in ALL. The most interesting finding is the presence of a large hsr (about the size of chromosome 11) at 11q21 in ~88% (21/24) of cells examined. The origin of this hsr waits for further molecular cytogenetic studies. In AML and MDS, hsr or dmin have been reported to be a result of amplification of AML1, MLL, C-MYC, or C-ETS1. To our knowledge, this is the first report on hsr in ALL.

Characterization of a familial RCC-associated t(2;3)(q33;q21) chromosome translocation. *J. Podolski^{1,2}, T. Byrski¹, S. Zajaczek¹, T. Druck³, D.B. Zimonjic⁴, N.C. Popescu⁴, G. Kata⁵, A. Borowska⁵, J. Gronwald¹, J. Lubinski¹, K. Huebner³.* 1) Hereditary Cancer Center, Dept. of Genetics and Pathology, Pomeranian Academy of Medicine, Szczecin, Poland; 2) Dept. of Human Ecology, University of Szczecin, Szczecin, Poland; 3) Kimmel Cancer Center, Jefferson Medical College, Philadelphia, PA; 4) Laboratory of Experimental Carcinogenesis, National Cancer Institute, Bethesda, MD; 5) Dept. of Urology, Postgraduate Medical Education Center, Warsaw, Poland.

We have identified a Polish family in which multifocal clear cell renal carcinoma segregates with a balanced constitutional chromosome translocation, t(2;3)(q33;q21), possibly with translocation positions similar to those of the renal cell cancer-associated t(2;3)(q35;q21) reported in a Dutch family. YAC and BAC contigs encompassing the 2q and 3q breakpoints were constructed and BACs crossing the breakpoints were partially sequenced. All known regional markers, genes and ESTs were mapped relative to the contigs, as well as to our breakpoint sequences. Two single ESTs map within the 2q breakpoint BAC, while the repeat rich 3q breakpoint region is gene poor. Physical mapping suggests that the 3q break is in 3q13, possibly near the border with 3q21 and is 10-20 megabases centromeric of the 3q21 breakpoint observed in the Dutch family with a renal cancer-associated t(2;3). Physical mapping illustrated that the 2q break is closely telomeric to the 2q31 FRA2G site, consistent with our G-band assignment. Molecular mapping data available for the Dutch 2q break was insufficient to determine similarity of the Polish and Dutch 2q breaks, although the cytogenetic data suggests different band positions. Characterization of full length cDNAs for the ESTs near the 2q break will determine if a gene(s) is altered by this familial RCC-associated chromosome translocation.

Application of molecular cytogenetic techniques for identification of the t(8;14)(q24;q32) translocation in a new case of non-Burkitt's lymphoma associated with numerous chromosome rearrangements. *S. Shekhter-Levin^{1,2,3,4}, D. Bahler³, L.C. Contis³, J.A. Pindzola³, K. Cumbie¹, M.E. Sherer^{1,2}, D. Kudla², U. Surti^{1,2,3,4}, S.M. Gollin^{1,2,3,4}.* 1) Pittsburgh Cytogenetics Lab, Magee Womens Hospital, Pittsburgh, PA; 2) Dept of Human Genetics, Univ of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; 3) Dept of Pathology, Univ of Pittsburgh School of Medicine, Pittsburgh, PA; 4) Univ of Pittsburgh Cancer Institute, Pittsburgh, PA.

The patient, a 51 yr old male, presented with diffuse adenopathy, hepatosplenomegaly and blasts in blood. Cervical lymph node histology suggested diffuse large B-cell lymphoma. Bone marrow pathology was also consistent with diffuse large B-cell lymphoma. Cytogenetic analysis of G-banded bone marrow cells revealed the following chromosome pattern: 41-42,X,-Y,add(8)(?q22),?der(8)t(8;14)(q24;q32),add(9)(q34),del(12)(p11.2p13.3),add(12)(p11.2),-13,-14,?der(14)t(8;14)(q24;q32)add(14)(p11.2),-15,-16,del(17)(p11.2),der(19)t(Y;19)(q11.2;p13.3), add(20)(q13.3),-21,+1-4mar[cp12]/46,XY[11]. Similar results were obtained on G-banded cells from the lymph node biopsy. Spectral karyotyping (SKY) revealed that the add(8)(q24) consists only of chromosome 8, and therefore, represents a duplication of part of 8q. Chromosome 8 painting confirmed the SKY findings regarding dup(8q) and indicated the presence of a chromosome 8 segment on distal 14q. The use of FISH with the MYC probe revealed two MYC signals on dup(8q), which was then redesignated as dup(8)(q22q24). A third MYC signal appeared on the distal long arm of the der(14), confirming the presence of the t(8;14)(q24;q32). The t(8;14)(q24;q32) is characteristic of Burkitt's lymphoma, but also has been reported in small noncleaved cell lymphoma and in posttransplant lymphoproliferative disorder. The interpretation of this finding in our patient with diffuse large B-cell lymphoma is unclear. The MYC gene may be involved in the unusually aggressive course of this lymphoma.

CLTC-ALK fusion in Inflammatory Myofibroblastic Tumor. *A.S Patel*¹, *E.J Perlman*¹, *C.M Coffin*², *C.A Griffin*¹.
1) Department of Pathology, John Hopkins University, Baltimore, MD; 2) University of Utah School of Medicine, Salt Lake City, Utah.

Inflammatory myofibroblastic tumors (IMT) are relatively rare tumors of the soft tissue occurring primarily in children and young adults. They are characterized by fascicles of myofibroblasts with a prominent inflammatory component of lymphocytes, eosinophils and plasma cells. We previously reported (Cancer Res., 59, 2776) rearrangement of the Anaplastic Lymphoma Kinase (ALK) gene in 3 IMTs using a dual color FISH probe (Vysis, Inc) surrounding the ALK gene. Subsequently using FISH analysis we have observed ALK rearrangements in 47% (9/22) of IMT cases. The most common fusion partner of ALK in anaplastic large-cell lymphoma (ALCL) is the nucleoplasmin gene (NPM) on 5q35, which results in a chimeric protein localizing to the nucleus. The IMT cases that had the ALK rearrangement were also positive for ALK staining by immunohistochemistry but the staining was predominantly cytoplasmic suggesting the ALK fusion partners in IMT may be different than those observed in ALCL. Frozen tissue from one IMT was used for 5' rapid amplification of cDNA ends (5'RACE) to identify the ALK fusion partner gene. 5'RACE identified clathrin heavy chain (CLTC) on chromosome 17q23 as the fusion partner of ALK. Additional IMT cases are being analyzed to determine other ALK fusion partners. These data provide conclusive evidence for ALK gene rearrangement in IMTs.

Spectral karyotyping (SKY) refines complex G-banded karyotypes in hematological malignancy. V. Sulcova, L. Parsons, A. Anguiano, B. White, K. Reddy. Cytogenetics, Quest Diagnostics Inc., San Juan Capistrano, CA.

The bone marrow cultures from hematological malignancies frequently yield poor quality GTG-metaphases and provide only partial information for many rearrangements. Therefore, we assessed the applicability of SKY to analyze complex bone marrow cases. Among the ten hematological malignancies investigated using SKY the known indications were one patient each with thrombocytopenia, chronic lymphoproliferative leukemia, and myelodysplasia, two patients had acute myeloid leukemia and 6 patients had lymphoma. Since, SKY alone was found to be inadequate to resolve many of the clonal abnormalities in a complex lymphoma case, we resorted to test SKY as an adjunct to G-banded analysis on 10 cases with 4 or more chromosome abnormalities. In two cases, SKY was used because the poor quality of metaphases precluded G-banded analysis. Two to seven metaphases were analyzed by SKY. Of the GTG-chromosome aberrations, 23% [22/95] were confirmed, 62% [62/95] were refined (incomplete or incorrect by GTG), 11% [5 clonal and 6 in one metaphase/95] new abnormalities were identified and 3% [3/95] were not found by SKY. Some of the interesting findings following SKY were the detection of *dm1* from chromosome 8, and *der(14)t(11;14)* in a mantle cell lymphoma case which had G-banded metaphases that could not be analyzed. An *hsr* on chromosome 7 (negative for *c-MYC*, *n-MYC*, *Her-2neu*, *BCR/ABL* and *p53*) was shown by SKY and FISH with *MLL* probe to have 4 copies of *MLL-11q23* region. SKY proved to be a powerful tool to further unravel complex G-banded karyotypes in hematological malignancies. A better understanding of the chromosome abnormalities using SKY will enhance our knowledge of diagnosis, prognosis and therapeutics.

Characterization of Askin tumor cell line, JK-GMS. *G.J Kim^{1,2}, S.H Park^{1,2}, S.Y Park², S.Y Cho³, M.J Lee³, H.S Ahn³, H.Y Shin³, J.G Chi³, C.J Kim³.* 1) Graduate School of Biotechnology, Korea University, Seoul; 2) Institute of Human Genetics, Dept. of Anatomy, Korea University College of Medicine, Seoul; 3) Dept. of Pathology and Pediatrics, Seoul National University College of Medicine, Seoul, Korea.

Askin tumor is a small round cell tumor in the thoracopulmonary region, which belongs to peripheral primitive neuroectodermal tumor (PNET) and Ewing's sarcoma (ES). PNET and ES are currently regarded as identical tumors sharing common chromosomal translocation, t(11;22)(q24;q22). TrkA mediates critical effects during human nervous system development including neural crest and also is known to modulate key biological features of brain tumors and neuroblastoma. Recently, we established Askin tumor cell line, JK-GMS, which expressed high level of trkA. For the characterization of this cell line, we analyzed the chromosomal aberrations and investigated the biological effects of trkA activity on the growth and differentiation of JK-GMS. JK-GMS had hypodiploid and showed chromosomal translocation, t(11;22)(q24;q22). Seven kinds of structural clonal aberration were effectively analyzed by cross species color banding (RxFISH) and chromosome painting. The activation of trkA induced differentiation and inhibited growth of JK-GMS, while treatment with K-252a, an inhibitor of trk tyrosine kinase, abrogated the effects of NGF on growth and differentiation of JK-GMS. These results were quite similar to those of neuroblastoma cell lines and other childhood tumor cell lines of neural crest origin. From these results, it may be suggested that JK-GMS is a good model to investigate the therapeutic target for the treatment of PNET/ES.

Analysis of Genetic Alterations in Primary Nasopharyngeal Carcinoma by Comparative Genomic Hybridization

(CGH). *L. Sun¹, Y. Fong², J.S.T. Sham³, Y. Guo², M. Deng², Q. Liang², H. Zhang⁴, H. Zhou⁴, H. Tideman¹, J.M.*

Trent⁴, X.-Y. Guan³. 1) Oral Maxillofacial Surgery, The University of Hong Kong, Hong Kong, PR China; 2) Cancer Center, Sun Yat-sen University of Medical Sciences, China; 3) Department of Clinical Oncology, The University of Hong Kong, Hong Kong; 4) Cancer Genetics Branch, NHGRI, NIH, Bethesda, MD.

To identify genetic alterations associated with the development and progression of human nasopharyngeal carcinoma (NPC), 57 tumors were analyzed using comparative genomic hybridization (CGH). In 47 cases chromosomal imbalances were found. Several recurrent chromosomal abnormalities were identified in the present study. The most frequently detected chromosomal gains involved chromosomes 12q (24 cases, 51%), 4q (17 cases, 36%), 3q (16 cases, 34%), 1q (15 cases, 32%), and 18q (15 cases, 32%). Common regions of gain involved 12q13-q15, 4q12-q21, and 3q21-q26. High copy number increases of chromosomal materials were detected in 4 chromosomal regions, 3q21-q26.2, 4p12-q21, 8p, and 12q14-q15. The most frequently detected loss of chromosomal materials involved chromosomes 16q (26 cases, 55%), 14q (21 cases, 45%), 1p (20 cases, 43%), 3p (20 cases, 43%), 16p (19 cases, 40%), 11q (17 cases, 36%) and 19p (16 cases, 34%). The most common regions of loss involved 14q24-qter, 1pter-p36.1, 3p22-p21.3, 11q21-qter, and the distal region of 19p respectively. Genomic alterations detected using CGH were compared and found to be largely consistent with those identified using banding analysis and loss of heterozygosity studies. However, several previously unrecognized recurrent alterations were also identified in the present study including: gain of 4q and 18q, and loss of 16q, 14q, and 19p. In addition, gain of 1q, 8q, 18q, and loss of 9q showed statistically significant association with advanced clinical stage ($p < 0.05$). Identification of recurrent sites of chromosomal gain and loss identify regions of the genome that may contain oncogenes or tumor suppressor genes respectively which maybe involved in the tumorigenesis of NPC.

Recurrent Chromosome Changes in 31 Primary Ovarian Carcinomas Detected by Comparative Genomic

Hybridization. *T.C.M. Tang¹, J.S.T. Sham¹, Y. Fong², L. Sun³, L.X. Qin⁴, X.-Y. Guan¹*. 1) Clinical Oncology, The University of Hong Kong, Hong Kong, China; 2) Cancer Center, Sun Yat-sen University of Medical Sciences, Guangzhou, China; 3) Oral Maxillofacial Surgery, The University of Hong Kong, Hong Kong; 4) Liver Cancer Institute, Zhongshan Hospital, Shanghai Medical University, Shanghai, China.

Ovarian cancer is one of the most frequent gynecological malignancies worldwide with poor prognosis. The development of new diagnostic, preventive, and treatment approaches requires good understanding of the mechanisms of the complex multi-step process of tumor pathogenesis in the ovarian cancer. Comparative genomic hybridization (CGH) has been applied to detect recurrent chromosome alterations in 31 primary ovarian carcinomas. Several nonrandom chromosomal changes including gains of 3q (17 cases, 55%) with a minimum gain region at 3q25-q26, 8q (16 cases, 52%), 19q (12 cases, 39%), Xq (11 cases, 35%), 1q (10 cases, 32%), 17q (10 cases, 32%), 12q (9 cases, 29%) with a minimum gain region at 12q12, and 20q (9 cases, 29%). High copy number gain (DNA sequence amplification) was detected in 10 cases. Amplification of 3q25-q26 and 12p11.2-q12 were detected in 4 and 3 cases, respectively. The regions most frequently lost included: 16q (9 cases, 29%), 1p (7 cases, 23%), 18q (7 cases, 23%), and 22 (7 cases, 23%). The recurrent gain and loss of chromosomal regions identified in this study provide candidate regions that may contain oncogenes or tumor suppressor genes respectively involved in the development and progression of ovarian cancer.

Cytogenetic analyses of six neuroblastoma cell lines with cross-species color banding. *S.Y. Park¹, G.J. Kim², K.B. Lee², Y.H. Kang¹, H. Kim^{1,2}, Y.H. Chun¹, S.H. Park^{1,2}.* 1) Institute of Human Genetics, Dept. of Anatomy, Korea University College of Medicine, Seoul, Korea; 2) Graduate School of Biotechnology, Korea University, Seoul, Korea.

The cytogenetic analyses of chromosomal aberrations became an essential method to identify the genes involved in the pathogenesis of cancers. Recently, the chromosome painting and cross-species color banding (RxFISH) in identifying marker chromosomes became useful techniques. Neuroblastoma is a pediatric malignant neoplasm of neural crest origin. The mechanisms contributing to the development of neuroblastoma are largely unclear, but non-random chromosomal changes, such as deletion of chromosome band 1p36, aberrations of 17q, and amplification of the *MYCN* oncogene, have identified over the past years. The purpose of this study was to establish, in detail, karyotypes of six neuroblastoma cell lines (SK-N-AS, SMS-KCNR, SK-N-MC, SK-N-SH, SH-SY5Y, and IMR 32) by G-banding, RxFISH, and FISH with chromosome painting probes. Each cell line had a variable number of numerical and structural aberrations. Deletion of 1p36 and a partial gain of chromosome 17 with the breakpoint on 17q21 were observed in four cell lines each. Chromosome 6, 15, 16, and 22 were commonly involved in structural abnormalities. Homogeneously staining regions or double minute chromosomes were confirmed in three cell lines, all of which were turned out to be amplification of *MYCN*. The chromosomal aberrations of the six neuroblastoma cell lines were effectively analyzed by RxFISH and multiple chromosome painting probes. The nonrandom rearrangements suggest candidate regions for isolation of genes related to neuroblastoma.

BRCA1 copy number in paraffin embedded cancer tissue samples of known BRCA1 and BRCA2 mutation carriers using a BRCA1 FISH probe. BRCA1 copy number in paraffin embedded cancer tissue samples of known BRCA1 and BRCA2 mutation carriers using a BRCA1 FISH probe. *S.R. Young¹, N. Kataoka^{1,2}, Z. Wang¹, H. Kato^{1,3}, W.T. Loging¹, J.R. Marks⁴, N. Lehman⁵.* 1) Dept OB/GYN, Univ South Carolina Sch Med, Columbia, SC; 2) Dept GYN/OB, Kyoto Univ JAPAN; 3) Dept OB/GYN, Nagoya City Univ Hosp, JAPAN; 4) Dept Surgery, Duke Univ Durham, NC; 5) Dept Pathology, Creighton Univ, Omaha, NE.

BRCA1 and BRCA2 genes, located on 17q and 13q, respectively, have been linked to hereditary breast cancer. Women inheriting a mutant form of either gene have up to a 70-80% lifetime risk of developing breast cancer. Both BRCA1 and BRCA2 are thought to function as tumor suppressor genes. LOH studies have shown that loss of the corresponding wild-type allele in a BRCA1 or BRCA2 mutation-carrying individual is associated with the development of malignancy. We have developed a FISH probe for the BRCA1 gene to study allele loss in archived, paraffin-embedded, tissue samples. Studies of five normal breast tissue samples, obtained during reduction mammoplasty, showed 5.8 to 11.8% of interphase cells having one BRCA1 signal. Four known BRCA1 mutation carrying malignant breast cancer tissue samples showed 30.8 to 51.4% of cells having one BRCA1 signal present in the interphase cells. In comparison, four known BRCA2 mutation carrying malignant breast cancer tissue sections revealed 25 to 30% of interphase cells as having one BRCA1 signal present. While there appears to be greater allelic loss of BRCA1 in BRCA1 cases compared to BRCA2 cases, the difference appears too small to confidently differentiate the two. A BRCA2 probe FISH study may be helpful in this regard. Numerous Southern blot studies have reported LOH at 17q in sporadic breast cancer. Our current FISH results support the concept that the BRCA1 gene, or the loss of the BRCA1 gene, is important in both hereditary and non-hereditary breast cancers. The use of FISH technology to evaluate allelic loss of BRCA1 and other tumor suppressors may prove of value in the study of other forms of cancer. It remains to be seen if FISH probes can differentiate BRCA1 and BRCA2 mutation bearing tissue samples.

Chromosomal abnormalities in colorectal polyps. *A.E. Rojas-Atencio, K. Urdaneta, P. Estrada, M. Soto-Alvarez, L. Borjas-Fajardo.* Unidad de Genetica, Universidad del Zulia, Maracaibo, Zulia, Venezuela.

The colorectal cancer is the second cause of death for cancer in the world, they usually begin as a benign polyp and many of them are never developed in cancer. The development of the cancer starting from a polyp you happens when it happens a mutation in the genetic code that controls the growth and repair of the cells. The objective of this work is to report the chromosomal anomalies found in 15 colorectales polyps The chromosome analysis was carried out through technical of short culture. A numeric anomaly was considered if this repeated at least 3 times and a structural one if it repeated at least 2 times. The chromosome analysis of the 15 polyps histology reported as benign adenoma, they revealed clonal chromosome aberrations in 11 of the 15 cases, seven of them presented specially lost numeric type anomalies of the chromosomes 8 and 22, other recurrent numerical change were +11 and +18, alone in a case an anomaly of structural type was observed that corresponded to a deletion of the short arm of the chromosome 7 [(del17p)]. The chromosome analysis of the polyps adenoma has been correlated by some authors with clinical and pathological parameters and the same ones could serve as a parameter suggestive presage of progression of the illness toward the metastasis.

Karyotypic analysis of non-tumorigenic and tumorigenic, human prostatic epithelial cell lines representing a tumor progression model. *P.D. Storto¹, G. Bice¹, D. Bello-DeOcampo², S. Quader², M.M. Webber².* 1) Dept Pediatrics/Human Develop, Michigan State Univ, East Lansing, MI; 2) Dept Zoology Michigan State Univ, East Lansing, MI.

RWPE-1, a human prostate epithelial cell line, has been transformed with MNU (N-methyl-N-nitrosourea) to generate four new cell lines (referred to as the MNU cell lines). The four MNU cell lines have been designated WPE1-2A22, WPE1-NB14, WPE1-NB11, and WPE1-NB26, in increasing order of their malignancy. The cell lines represent different stages of tumor progression, from non-invasive to invasive, and will serve as an interesting in vitro model of prostatic neoplasia formation.

We have studied the four MNU cell lines in regards to their cytogenetic makeup, i.e. we have analyzed the specific chromosomal abnormalities present in the cell lines. The cell lines contained both common and unique aberrations; all four MNU lines had lost copies of chromosomes Y and 22, and gained entire copies of chromosomes 5 and 20. Breaks occurred at chromosomal regions 7q22, 9q11, 11q13, 11q23, 11q25, and 18q12.2. All cells lines revealed loss of material from chromosomes 7 and 18 long arms, and duplication of material from chromosomes 9 and 11 long arms. Abnormalities not common to all cell lines were a deletion of chromosome 2 long arm material and a 13q isochromosome in cell line WPE1-2A22, a deletion of chromosome 1 long arm material and loss of chromosome 13 in cell lines WPE1-NB14 and WPE1-NB26, a duplication of chromosome 19 material in cell line WPE1-NB11, a break at region 19q21 and duplication of material from the long arm of chromosome 13 in cell line WPE1-NB26, and small unidentifiable markers in cell lines WPE1-NB11 and WPE1-NB26. Overall, the more tumorigenic cell lines appeared to contain a greater number of cytogenetic rearrangements. We discuss the significance of these cytogenetic abnormalities in terms of their prevalence in prostate cancer, and in regards to the locations of tumor-suppressor genes that may be involved in the chromosomal rearrangements.

Two unusual rearrangements in hematological disorders studied by RT-PCR and/or FISH. *H.E. Wyandt^{1,2}, R.V. Lebo^{1,3}, X.-L. Huang¹, B. Loose¹, L. Oshry⁴, L. Weintraub⁵*. 1) Ctr Human Genetics, Boston Univ Sch Medicine, Boston, MA; 2) Pathology, Boston Univ Sch of Medicine, Boston, MA; 3) Pediatrics, Boston Univ Sch of Medicine, Boston, MA; 4) East Boston Neighborhood Health Center, Boston, MA; 5) Hematology/Oncology, Boston Univ Sch of Medicine, Boston, MA.

Fluorescence in situ hybridization (FISH) reveals an unusual karyotype, 46,XX,inv ins(9;22)(q34; q11.2q11.2) in bone marrow (bm) from a 56 y.o. female with chronic myelogenous leukemia (CML). Probes for bcr and abl-ass (Vysis, Downers Grove, IL) revealed 98% of interphases with a bcr/abl fusion. Metaphases showed juxtaposition of the bcr signal distal to the abl signal on der(9) and no bcr signal on der(22). Partial painting for chromosome 22 (pcp22subtel, Rainbow Scientific, Windsor, CT) revealed the subterminal region of der(22) to be intact. The bcr region of der(22) was thus inserted into der(9). RT-PCR of extracted mRNA amplified a 305 bp fragment, corresponding to a M-bcr/abl 210 kDa fusion product. In order for this product to be transcribed, both bcr and abl must be inverted. The patient had a complete hematological response and a minor cytogenetic response (reduction to 70% percent cells with bcr/abl fusion) with interferon therapy. Bm from a 49 y.o. male with acute promyelocytic leukemia (APL) and associated disseminated intravascular coagulation has the karyotype, 46,XY,t(17;15;17) (q11;q22;q25). FISH using probes for PML in 15q22 RARA in 17q22 (Oncor, Gaithersburg, MD) revealed distal 17q and RARA region of a chromosome 17 to be fused to part of the PML region on a chromosome 15. Distal 15q including the distal part of PML is translocated to the 17 homolog (breakpoint in 17q25), and distal 17q is translocated to the proximal part of the first 17. This complex rearrangement resulted in an inverted partial PML/RARA fusion on der(15), loss of RARA from der(17) and partial PML and intact RARA signals on der(17). The patient was treated with all-trans-retinoic acid, daunorubicin and cytosine arabinoside. He is now in complete hematologic and cytogenetic remission. Both patients have thus responded to treatments suggestive of typical CML and APL, respectively, despite their unusual chromosomal rearrangements.

Cytogenetics finding in Breast Ductal Carcinoma. *M.L. Soto-Alvarez, A. Rojas-Atencio, F. Alvarez-Nava, K. Urdaneta, L. Gonzalez, A. Boscan.* Unidad de Genetica Medica, Universidad del Zulia, Maracaibo, Zulia, Venezuela.

The feminine breast cancer it is one of the old problems of public health in our society, from the past decade an improvement in him has been observed diagnosis, as well as also in the identification of parameters prognostic that permit a better evaluation of these patients. The objective of this work is to report, the chromosomal anomalies in 32 breast ductal carcinoma (BDC) . In this report, we present the chromosomal abnormalities found in 32 primary breast ductal carcinomas. The tumor samples were studied using the technique for short-term culturing and cytogenetic analysis with G-banding. Only one tumor with normal karyotype was observed. Thirty one (99%) of the tumors had chromosomal abnormalities including 21/ (65,6 %) in which chromosome 1 was involved (trisomy, monosomy or structural abnormalities of the type t(1q;2p) and del(1q42). Other recurrent anomalies such as del(12p); del 4(p); +7; +8; -7; -3; were observed. The significance of these findings and their role in tumorigenesis will become more evident with close follow-up of women who have tumors with an abnormal karyotype.

A “static” karyotype in epithelial cancer cell lines despite ongoing chromosomal instability. *A.V. Roschke¹, K. Stover¹, G. Tonon¹, A.A. Schaffer², I.R. Kirsch¹.* 1) Genetics Department, Medicine Branch, NCI, Bethesda, MD; 2) Computational Biology Branch, NCBI, Bethesda, MD.

Most human tumors and tumor cell lines exhibit numerical and structural chromosomal abnormalities. The goal of this study was to determine the ongoing rates of structural and numerical chromosomal instability in selected cancer cell lines and to investigate the consequences of these rates to karyotypic progression. Our approach was to make single cell subclones of two colorectal (HCT-116 and HT-29) and two ovarian (SKOV-3 and OVCAR-8) cell lines and to delineate rates over time of numerical and structural chromosome changes. We performed SKY, CGH and FISH with chromosome-specific centromeric probes on cell lines and their subclones. The karyotypes of all four cell lines showed evidence of genome destabilization at some previous moment or period in their history. While significant ongoing structural and/or numerical chromosomal instability could be demonstrated in all cell lines during our period of observation, there was a relative stability of the consensus karyotype over many generations. No new clonal structural chromosomal reconfigurations emerged and the few numerical changes of karyotypes were restricted to losses of abnormal chromosomes. This implies a kind of genomic optimization under the present conditions of cell culture and suggests a link between genomic stabilization and cell propagation. We have been able to support this possibility by computer modeling.

Physical and hematopoietic transcript map of a 5q31 "critical subregion" associated with the 5q- syndrome. S. Kamakari¹, V. Konstantinopoulou¹, N.P. Anagnou^{1,2}. 1) Institute of Molecular Biology and Biotechnology; 2) University of Crete School of Medicine, Heraklion, Greece.

The 5q- syndrome represents a preleukemic state, exhibiting an acquired interstitial 5q23-31 deletion. Four critical subregions seem to be involved in leukemogenesis. To delineate the role of one of these subregions, we constructed a YAC contig between the GM-CSF/IL-3 and TCF-1 genes. Extensive PCR screening of the CEPH and ICI YAC libraries, resulted in the isolation of twelve YACs: three YACs (854G6, 679B9, 14DG10) positive for the GM-CSF/IL-3 genes, five YACs (624G4, 969H4, 969G11, 698D8, 12BE7) positive for the TCF-1 gene and four YACs (14BA6, 15AE2, 14EB12, 28CB1) positive for seven STSs (5157S1, 5250S, bac5060S, bac5177S, 5322S, 5342T and 5328S). STS content mapping of all twelve YACs resulted in the construction of the first complete YAC contig of this subregion and documented that the GM-CSF/IL-3 and TCF-1 genes are linked. The precise size of the genomic region is being investigated by generating a long-range restriction map. Furthermore, in order to develop a hematopoietic transcript map of the subregion, twenty-six chromosome 5-specific ESTs were selected, on the basis of their origin and assignment. PCR analysis and *in silico* searches, resulted in sublocalisation of nine ESTs to the identified YACs and documented their expression in a bone marrow cDNA library. Two of them, namely T77830 and W92884, mapping proximal and distal to the IL3 gene, respectively, were further analysed. Human RNA dot blot analysis of the EST T77830, exhibited expression in kidney, liver, small intestine and lung. Northern blot analysis also documented its expression in a variety of tissues, including bone marrow, with a transcript size of approximately 4 kb. Following the technique of 5' and 3' rapid amplification of cDNA ends (RACE) for this EST, using a bone marrow cDNA library as a template, two overlapping clones, covering a total length of 3.8 kb were isolated and sequenced. Characterisation of the full-length cDNA is in progress. RACE amplification of the EST W92884 resulted in a 500 bp PCR product, currently used as a probe in a bone marrow cDNA library hybridisation screening.

Non-random X-inactivation suggests that juvenile hemangiomas are monoclonally derived. *J.W. Walter¹, P.E. North², A. Mizeracki², M. Waner³, D.A. Marchuk².* 1) Department of Genetics, Duke University Medical Center, Durham, NC; 2) Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR; 3) Department of Otolaryngology, University of Arkansas for Medical Sciences, Little Rock, AR.

Juvenile hemangiomas are the most common tumors of infancy, and occur in as many as 10% of all births. These benign vascular lesions enlarge rapidly during the first year of life by hyperplasia of endothelial cells and attendant pericytes, then spontaneously involute over a period of years, leaving a paucicellular fibrofatty residuum. In the present study, six sporadic proliferative-phase hemangiomas were collected following surgical resection, and dissected to enrich for the endothelial cell component of each sample.

To determine if hemangiomas represent a monoclonal expansion from a single progenitor cell, we assayed X-inactivation patterns for each lesion (all were obtained from female patients) using the polymorphic X-linked human androgen receptor-A (HUMARA) locus. Three of six hemangiomas, all from patients less than five months of age, demonstrate a significant degree of allele loss, in one case almost complete, following methylation-sensitive restriction digest and PCR amplification, implying a non-random X-inactivation pattern, and a monoclonal origin to one or more cellular components of the lesion.

These results suggest that one or more genetic events in a single cell may contribute to hemangioma development. Eventually, accumulation of non-clonally derived cells would mask the clonal nature of these lesions. The identification of clonal, proliferative samples provides an opportunity to characterize genetic events that may precede or modulate hemangioma development, and to identify genes involved with hemangioma formation.

Characterization of a Complex Chromosome Rearrangement involving 6q in a Melanoma Cell Line: Isolation of a Candidate Tumor Suppressor Gene Interrupted by the Breakpoint at 6q16. *X.Y. Guan¹, H. Zhou², J.S.T. Sham¹, H. Zhang², J.M. Trent².* 1) Dept Clinical Oncology, Univ Hong Kong, Hong Kong, China; 2) Cancer Genetics Branch, NHGRI, NIH, Bethesda, MD.

The incidence of human malignant melanoma has increased dramatically in many parts of the world. Deletion of 6q is one of the most frequent chromosomal alterations in malignant melanoma with a breakpoint cluster at 6p11-q21. Recently, we used G-banding analysis and micro-FISH technique to detect a complex chromosomal rearrangement involving 6q and 17p in a melanoma cell line UACC-930. The rearrangement includes an inversion involving 6q, inv(6q;6q)(q16;q27), and a translocation involving the inverted 6q and 17p13. A BAC clone covering the breakpoint 6q16 was isolated by FISH screen. A novel gene (named BIM1, broken in melanoma 1) interrupted by the breakpoint was isolated by partial sequencing analysis of the BAC clone. Full-length cDNA of BIM1 with two isoforms has been isolated. Sequence analysis identified a significant similarity of BIM1 and prenyl transferase gene, which is required for the cancer-causing activity of Ras gene. Loss of BIM1 was also detected in 10/12 melanoma cell lines. Our results indicate BIM1 may play an important role in the development of malignant melanoma.

Parental Origin of 1p36 Deletions: Does Size Matter? *H.A. Heilstedt, W.L. Matheny, L.G. Shaffer.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

We have characterized a total of 41 patients with deletions of chromosome 1p36 using microsatellite polymorphisms and probes for fluorescence in situ hybridization (FISH). Clinical features of this syndrome include mental retardation, growth delay, hypotonia, deafness, and facial dysmorphism. The majority of deletions are maternal in origin (64%), while 28% are paternal and in 8% the parental origin could not be determined. Interestingly, the majority of patients with the largest deletions are paternal in origin. Frequent occurrence of deletions of distal chromosome 1p has been found in neuroblastoma (NB). There appears to be a distinction between the deletion size in those tumors with MYCN amplification and those without, in those tumors with MYCN amplification tend to have larger deletions of 1p. Subsequently, there is a suggestion that there are multiple tumor suppressor loci on 1p. Previously, loss of heterozygosity studies in MYCN single copy tumors have shown the lost 1p36 allele is usually of maternal origin suggesting that imprinting may play a role in the development of NB. The critical region for one of the proposed NB tumor suppressor genes is between D1S214 and D1S244 (~7cM). Of the 10 patients with deletions breakpoints proximal to D1S214, six (60%) are paternal in origin, 3 (30%) are maternal and one (10%) parental origin could not be determined. None of these patients have developed neuroblastoma. The average age of these patients is 9.3 years (range 18 mo. to 19.3 yrs.), beyond when neuroblastoma is usually diagnosed, lending further support to a possible role for imprinting. KIAA0591 is a novel kinesin-related protein, which had been mapped to this critical region by radiation hybrid mapping. It has been proposed to function as a tumor suppressor in an epigenetic manner as no mutations were found when NB tissue was examined. Using a BAC, which contains the 3' end of this gene, we have more precisely mapped KIAA0591 relative to this critical region using our panel of deletion patients and FISH. The parental origin and size of deletion are proving to be helpful tools in delineating the molecular basis of neuroblastoma.

In Vivo Hypermutability Associated with a De Novo Interstitial Deletion Adjacent to the Glycophorin A Gene on Chromosome 4. *S.G. Grant*¹, *S.L. Wenger*². 1) Center for Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA; 2) Department of Pathology, West Virginia University, Morgantown, WV.

Somatic mutation is a basic mechanism of carcinogenesis and aging, and is influenced by both genotoxic exposure and genetic predisposition. The human in vivo glycophorin A (GPA) assay quantitates loss of expression of one allele of the gene responsible for the MN blood type at 4q28-31.1. Elevated frequencies of GPA variants have been documented in blood samples taken from individuals exposed to ionizing radiation or genotoxic chemicals, or from patients with hereditary cancer-prone or premature aging syndromes. In this study, the frequency of GPA allele-loss variant erythrocytes was found to be unusually high in a pediatric patient with a de novo interstitial deletion of 4q25q27. The magnitude of the observed increased variant cell frequency, 30-fold over age-matched controls, would be consistent with an acute whole body dose of ionizing radiation of ~6.5 Gy, or the spontaneous variant cell frequency measured in the cancer prone syndrome Fanconi anemia. Mechanistically, these results are similar to those previously documented in a cell culture system, in which a 100-fold increase in the frequency of mutation at the CHO aprt gene was associated with translocation of an allele adjacent to an inversion breakpoint. The translocated allele was highly susceptible to spontaneous deletion, and was also found to easily undergo de novo gene inactivation, perhaps through a position effect. The possibility of unresolved genetic instability in the breakpoint regions of chromosome rearrangements has important implications both for hereditary disease in the form of contiguous gene syndromes, and for somatic disease in the form of continuing hypermutability associated with the genomic rearrangements characteristic of carcinogenesis.

Comperative Genomic Hybridization (CGH)in peripheral blood of Polycythemia Vera and essential thrombocytosis patients. *A. Amiel¹, Y. Harishano², M.D. Fejgin¹, E. Gaber¹, M. Lishner².* 1) Genetics Unit, Meir General Hosp, Kfar-Saba, Israel; 2) Department of Medicine, Meir General Hospital.

Background- Polycythemia Vera (PV) and Essential Thrombocytosis (ET) are chronic clonal myeloproliferative diseases originating from multi-potent stem cell. Om bone marrow examination chromosomal abnormalities are encountered in 15-43% of PV patients and 5% of ET patients. However, no specific abnormality has been described. Cytogenetic abnormalities are more common in both diseases when a transformation of the disease occurs or following myelosuppressive treatment. Cytogenetic analysis in these diseases is usually done utilizing simple chromosomal karyotyping or by FISH. The aim of this research was to study cytogenetic aberrations associated with these diseases by CGH analysis. Methods- 12 PV and 8 ET patients participated in the study. Seven of the PV group were treated by phlebotomy, 12 were treated with hydroxyurea and 3 patients recieved P32 and/or Busulfan at different periods of time during their disease. All ET patients were treated with hydroxyurea. CGH analysis of the peripheral blood was performed using the method described by Kallioniemi et all (1992). Results- 3 PV patients (25%) had an abnormal karyotype: One had a gain in 11q12, one had combination of a gain in 11q12, 6p21.1-22 and 17q12-21, and another patient had a deletion 7q11.2 . Only one ET patient (12.5%) had again in 18p. Conclusion- Peripheral blood analysis by CGH in PV and ET patients did not reveale the commonly reported cytogenetic aberrations. However, other changes were observed, in particular a gain in 11q12 in treated PV patients.

Lack of spatial preference between the der(BCR/ABL) and the der(ABL/BCR) in CML interphase cells. *R.M. Chandra, M.J. Macera, R.S. Verma.* Department of Medicine, Wyckoff Heights Medical Center, Brooklyn-New York Hospital/Weill Medical College of Cornell Medical Center, New York, NY.

Do chromosomes involved in translocations have any special arrangement at interphase? It has been suggested that chromosomes 9 and 22 remain in close proximity in the center of interphase nuclei, a situation which enhances exchanges between them. This is of interest because of the t(9;22)(q34;q11.2) translocation, resulting in the Philadelphia (Ph) chromosome, the hallmark of CML. However, still unknown is whether, after the translocation occurs, the der(9q34) region remains closer to the Ph chromosome than the normal 9q34 region, and whether the two derivative chromosomes remain closer than the two normal chromosomes. The BCR/ABL translocation probe had been used to identify the normal BCR, the normal ABL, and the BCR/ABL rearrangement, but identification of the der(ABL) region had been difficult. Recently, a BCR/ABL probe (Vysis) became available in which the ABL label is extended 5' from exons 5-11 to an area containing sequences specific for the argininosuccinate synthetase gene (ASS). When this probe is applied to a Ph-positive CML sample, the der(9q34) can be identified as an additional red signal, smaller than the normal 9q34 signal. This probe was applied to two cases of CML which both had the t(9;22)(q34;q11.2) in 100% of analyzed metaphases. One hundred bone marrow nuclei were examined per case. The distance from the fused BCR/ABL (yellow) signal was measured to both the der(ABL) (small red) signal and the normal ABL (large red) signal and compared. Also, the distance from the normal ABL signal to the normal BCR (large green) signal was measured and compared to the distance from the BCR/ABL to the der(ABL). No preference was observed for either of the comparisons in either case. This suggests that in interphase cells, once the BCR and ABL genes have translocated to form the chimeric BCR/ABL gene, there does not appear to be further selection keeping the translocated regions closer than their normal homologues.

HER2/neu analysis of paraffin-embedded testicular germ cell tumors using fluorescence in situ hybridization.

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The HER2/neu oncogene, located on chromosome 17q, encodes a tyrosine kinase receptor which is homologous to the human epidermal growth factor receptor. Previous studies using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) on paraffin-embedded breast tumors have correlated HER2/neu overexpression and amplification with poor prognosis and early relapse. Recent evidence has shown a decrease in disease aggressiveness and in increase in response rate (Ross and Fletcher, 1998) in patients positive for HER2/neu amplification that were treated with the monoclonal antibody rhuMAB HER-2 (Herceptin).

The incidence of testicular germ cell tumors (TGCT) commonly found in men between the ages of 15 and 34 have more than doubled over the past four decades warranting the need for increased detection and treatment. Currently, there is an 80% survival rate for patients with newly diagnosed and treated TGCT. The remaining 20% either do not respond to treatment or are candidates for early relapse. A current IHC study on paraffin-embedded TGCT detected 15% HER2/neu protein overexpression overall. To date there are no data for HER2/neu analysis on TGCT utilizing FISH. We present preliminary FISH data from a blinded study on 64 of the same paraffin-embedded TGCT as the aforementioned IHC study. A total of forty nuclei were scored per tumor. Of the 64 tumors analyzed, 62 (96.87%) averaged 2.1 signals/nuclei (2s.d. = 1.04; 95% C.I. = 0.0089) and were considered normal. Two tumors (3.125%) exhibited HER2/neu amplification with an average of 5.9 signals/nuclei (2s.d. = 0.14; 95% C.I. = 0.006) and were scored as amplified. Future work will be twofold: to determine whether a correlation exists between HER2/neu protein overexpression (IHC) and DNA amplification (FISH) in TGCT and to determine whether this overexpression/amplification correlates to resistance to treatment and early relapse.

Equivocal cases of Her-2 gene amplification detected by fluorescence in situ hybridization. *F.P Espinoza¹, X. Yang², K.S. Reddy².* 1) Dept Pathology, Quest Diagnostics, San Juan Capist, CA; 2) Dept Cytogenetics.

Her-2 gene amplification was detected using FISH on paraffin embedded tissue sections. Among 424 mammary carcinomas studied for Her-2 gene amplification, 74 (17.5%) were positive, 335 were negative and 15 [~3.5%] were equivocal using PathVysion FISH (Vysis Inc) assay. The ratio of the Her-2 signals to the centromere 17 signals (which maps to 17q11.2q12) when equal or ≥ 2 was considered positive. The equivocal cases were those with a ratio < 2 but with a subpopulation of cells having Her-2 amplification equal or ≥ 2 . Some of these cases also had chromosome 17 aneuploidy. Other cases had borderline value of 1.8-2.0. One tumor showed amplification in a restricted region and had a Her-2 ratio of 1.33. These cases were examined using immunohistochemistry (IHC) method. The FISH and IHC findings were compared. The IHC Dako antibody staining when weakly positive was scored as a 2+; and when strongly positive was a 3+; . Lack of or faint staining was considered negative (0 or 1+). Six tumors with a Her-2 gene amplification ratio < 2 had a subpopulation of cells (30-50%) with Her-2 ratio ≥ 2 . Two such tumors with the largest population (49% and 50%) of amplified cells were 2+ positive by IHC. Four cases with a Her-2 gene ratio < 2 and aneuploid for chromosome 17 in the majority of cells also had a subpopulation of cells (12.5% to 40%) with ≥ 2 Her-2 ratio. Two such tumors were positive (3+ or 2-3+) by IHC. Three borderline cases with a 1.8-2.0 ratio for Her-2 gene were negative 1+ by IHC. FISH evaluation of Her-2 amplification is straightforward in the majority of cases. However, it is in tumors with a Her-2 ratio < 2 and a mosaic pattern of amplified and normal cells that caution must be exercised by evaluating these cases with an IHC assay. In this study, 26% (4/15) of tumors with an equivocal FISH result were found to be IHC positive. If the equivocal cases were considered as negative based solely on the equal or < 2 Her-2 gene ratio, roughly 1 per 117 cases would have been false negative considering a 90% correlation between FISH to IHC results.

Somatic Mutational Evidence for Tissue-specific Premature Aging in the Bone Marrow of Patients with Sickle Cell Hemoglobinopathy. *B.M. Luccy¹, L. Saglammer¹, W.L. Bigbee¹, J.D. Hord², S.G. Grant¹*. 1) Center for Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA; 2) Division of Hematology/Oncology, Akron Childrens Hospital, Akron, OH.

Sickle cell hemoglobinopathy is a molecularly simple genetic disorder with complex pleiotropic effects on many tissues. One of the most severely affected is the bone marrow, which must make up for constant hemolysis-induced anemia by increased processing and releasing of erythrocytes into the circulation. We have investigated whether this increased mitotic activity in the bone marrow results in higher frequencies of somatic mutation in circulating blood cells. Somatic mutation, an intrinsic factor in aging and carcinogenesis, can be measured in peripheral blood erythrocytes using a flow cytometric assay based on the glycophorin A (GPA) erythrocyte surface protein. GPA variant cell frequencies were analyzed in a group of 22 informative pediatric sickle cell patients. Mutation frequencies in the patient population were correlated with age and significantly higher than matched disease-free controls. Moreover, mutation frequencies correlated with clinical indicators of disease severity, including white blood cell count, reticulocyte count and blood hemoglobin. These results imply that the bone marrow of sickle cell patients has a somatic mutational burden characteristic of an older normal population, and that they may be at increased risk of hematopoietic malignancies. Perhaps more importantly, this data provides in vivo evidence that induction of cell replication (mitogenesis) directly increases the endogenous mutation frequency and/or susceptibility to genotoxic agents.

High resolution deletion mapping defines a 3-cM region of 15q loss in human malignant mesothelioma. A. De Rienzo¹, B.R. Balsara¹, S.C. Jhanwar², J.R. Testa¹. 1) Human Genetics, Fox Chase Cancer Center, Philadelphia, PA; 2) Human Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY.

Malignant mesotheliomas (MMs) are highly aggressive neoplasms that arise primarily from the surface serosal cells of the pleural, peritoneal, and pericardial cavities. Epidemiological studies have established that exposure to asbestos fibers is the primary cause of MM. The accumulation of multiple, recurrent cytogenetic deletions in most MMs suggests a multistep process in this malignancy, which is characterized by the loss and/or inactivation of several tumor suppressor genes (TSGs). Earlier karyotypic studies of MMs demonstrated frequent deletions of specific regions within chromosome arms 1p, 3p, 6q, 9p, 13q, and 22q. Recently, we reported comparative genomic hybridization analyses that enabled us to identify a new recurrent site of chromosomal loss within 15q in this malignancy (Balsara et al., Cancer Res. 59: 450-454, 1999). In addition, loss of heterozygosity analysis, using 8 MM cases and 16 polymorphic microsatellite markers, identified a minimal region of chromosomal loss at 15q11.1-15. To more precisely map the region of 15q deletion, we have now constructed a high-resolution map of 15q loss in 46 MM cell lines using 26 polymorphic DNA markers spanning the entire long arm. This study has defined a shortest region of overlap (SRO) within an ~3-cM region in 15q15.3. Fluorescence in situ hybridization analysis, using YACs localized to 15q15, confirmed losses from this region in several MMs with small 15q deletions. cDNA microarrays of ESTs mapped to the SRO are currently being utilized to identify candidate TSGs downregulated in MM.

Cloning of a recurrent site of chromosome translocation in cutaneous melanoma. *K.M. Brown^{1,2}, D.A. Stephan^{1,3}, J. Zhang³, M. Heiskanen³, G. Bethel⁴, C.R. Robbins³, C. Carder⁴, A. Mungall⁴, P.S. Meltzer³, J.M. Trent³.* 1) Res Center for Genet Medicine, CNMC, Washington, DC; 2) GWU Genetics, Washington, DC; 3) NHGRI/NIH, Bethesda, MD; 4) Sanger Centre, Cambridge, UK.

UV radiation plays a key role in the development of melanoma via the generation of photo-adducts, but the genetic mechanisms behind melanoma progression have yet to be clearly elucidated. A large body of evidence implicates chromosome 6q as a major genetic determinant for melanoma. Partial loss of 6q has been observed in the majority of malignant melanomas, while translocations in the 6q12-6q21 region have been observed in still more tumors. In addition, transfer of human chromosome 6 into melanoma cell lines has been shown to reverse the tumorigenic phenotype. This strongly argues for the presence of a tumor suppressor gene whose deletion is critical for the development or progression of melanoma.

We are currently mapping the breakpoint of a nonreciprocal translocation [t(1;6)(q21;q14)] found in the HA-A melanoma primary cell line. Previously, we identified a single YAC clone spanning the breakpoint (Zhang et al., 1995). We have since identified BAC clones by STS content from this YAC and used them for fiber-FISH on the HAL-26 cell line (contains the translocated chromosome in a rodent background) together with a chromosome 1 BAC flanking the breakpoint. The results showed a gap of ~200kb between one chromosome 6 BAC and the chromosome 1 BAC. To span the breakpoint, we end-sequenced and walked in both directions from the chromosome 6 BAC, fingerprinted it, and integrated it into the Sanger Centre chromosome 6 BAC contig. The BAC integrated into a large contig that extends >200kb in either direction from the chromosome 6 BAC. We are currently identifying the BAC clone that spans the breakpoint by fiber-FISH, end-STS content mapping, and end-STS Southern blotting in HAL-26. Identification and sequencing of the BAC that contains the gene interrupted by the translocation will hopefully identify a previously unrecognized tumor suppressor gene and allow us to better understand the molecular pathophysiology of this common malignancy.

Low incidence of follicular lymphoma and translocation t(14;18)(q32;q21) by PCR analysis in Ecuador, South America. *P.E. Leone^{1, 2}, J.C. Pérez¹, M.E. Sánchez^{1, 2}, M. Arévalo¹, C. Paz-y-Mino^{1, 2}*. 1) Hum Mol Genet & Cytogen Lab., Department of Biological Sciences; 2) Medicine Faculty, Catholic University of Ecuador, Quito-Ecuador.

The chromosomal translocation t(14;18)(q32;q21) has been shown in diagnostic tissue specimens from approximately 85% of patients with follicular and 30% with diffuse non-Hodgkins lymphomas (NHL). This translocation leads to an overproduction of the Bcl-2 protein on the basis of increased Bcl-2 mRNA levels which interferes with normal apoptosis of B-lymphocytes. The breakpoints on chromosome segment 18q21 (BCL-2) have been shown to cluster at two main regions 3': approximately 70% occur within the major breakpoint region (mbr) and up to 20% are found within minor breakpoint region (mcr). There is evidence that the distribution of NHL subtypes differs by geographic region, however there is not data for Ecuador. Using PCR, we have examined the frequency of this translocation (mbr and mcr) in 65 NHL tumors samples obtained during five years in the principal hospitals of Quito-Ecuador. Of 65 NHL only 5 were follicular lymphoma, which represents an incidence of 7.7%. This incidence is quite lower than one in North America (30%). In addition, we do not find the translocation in the samples analyzed, which was confirmed by use of a control PCR; probably, then, this translocation is not common in NHL in Ecuador. These findings confirm that there are differences in the incidence of specific subtypes of NHL across some geographic areas and suggest that genetic factors may be responsible for the observed differences.

The t(4;11)(q21;p15) - a unique recurring structural abnormality associated with ALL, AML as well as T-cell lymphomas. *M. Thangavelu¹, B. Huang¹, K. Richkind².* 1) Genzyme Genetics, Orange, CA; 2) Genzyme Genetics, Santa Fe, NM.

The t(4;11)(q21;p15) is a relatively rare rearrangement involving the same band on chromosome 4 as the t(4;11)(q21;q23), an abnormality most frequently associated with ALL in very young patients and poor prognosis. The t(4;11)(q21;p15) has been reported in ALLs (some with myeloid surface markers) a case of AML and a biphenotypic leukemia. We present a case of T-cell lymphoma and one of AML with this rearrangement. The clinical history in the latter is suggestive of a secondary leukemia. These are the first reported cases of t(4;11)(q21;p15) in a T-cell lymphoma and therapy induced secondary leukemia. One difference between the t(4;11)(q21;q23) and the t(4;11)(q21;p15) appears to be that while the former is most frequently observed in very young patients, the latter is observed in older male children and young to middle aged male patients. Our cases along with those previously reported suggest that the t(4;11)(q21;p15) may be a unique recurring structural abnormality associated with a variety of hematologic malignant disorders, both primary as well as secondary. The case of T-cell lymphoma reported here suggests that this may be the first translocation associated with ALLs, AMLs as well as lymphomas. Investigations at the molecular level are essential in determining if the breakpoint on chromosome 4 involves the AF-4 gene as in t(4;11)(q21;q23), in learning more about the sequences on 11p15 involved in this rearrangement as well as determining if the rearrangements in the various disorders, which appear similar at the microscopic level, are identical at the molecular level.

Computational Methods for Gene Expression Based Tumor Classification. *W. Li^{1,2}, M. Xiong¹*. 1) Human Genetics Center, University of Texas-Houston, Houston, TX; 2) Beijing Institute of Basic Medical Sciences, P.O.Box 130(3), Beijing, 100850, P.R.China.

There is increasing interests in changing the emphasis of tumor classification from morphologic to molecular. Gene expression profiles may offer more information than morphology and provide an alternative to morphology-based tumor classification systems. In this report, We have investigated the following methods: fish's linear discriminant function, neural network and support vector machine methods for gene expression based tumor classification, and we emphasize the following key issues such as feature variable selection and stability analysis. In the selection of feature variables, principal component analysis, stepwise optimization, Monte Carlo simulation and exhaustive searching methods were used. For stability analysis, the method of sample random permutation was used to get the comprehensive classification accuracy. Finally, in order to demonstrate the reliability of our method and program, the proposed tumor classification system was applied to three data sets: colon cancer data including 22 normal and 40 colon tumor tissues, breast cancer data including 14 human mammary epithelial cells and 13 breast tumors, and human acute leukemia data including 47 acute lymphoblastic leukemia (ALL) and 25 acute myeloid leukemia (AML) samples. Through these three examples, we show that using two or three genes can achieve more than 90% accuracy of classification. This result implies that after initial investigation of tumor classification using microarrays, a small number of selected genes may be used as biomarkers for tumor diagnosis. Further we also showed that stepwise optimization method based on Fisher's linear discriminant function is a practicable method for gene expression-based tumor classification.

DIFFERENTIAL GENE EXPRESSION IN COLORECTAL TUMORS. *N.A. Pinheiro¹, L.F.L. Reis¹, S.J. Souza^{1,3}, F. Soares³, S.D. Pena^{2,3}, A.J.G Simpson¹.* 1) Cancer Genetic, Ludwig Institute , Sao Paulo, SP., Brazil; 2) Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais, M.G., Brazil; 3) Hospital do Cancer - A.C.Camargo, Sao Paulo, S.P., Brazil.

Colon tumorigenesis is considered a complex multistep process that occurs through a series of gene mutations, leading from precursor lesions to invasive and metastatic adenocarcinoma. The development and progression of cancer and the reversal of tumorigenicity are accompanied by complex changes in patterns of gene expression. Microarrays of cDNA provide a powerful tool for studying these complex phenomena. The purpose of the present study has to determine differential gene expression in colon tumors as compared with normal tissue. We also investigated the possible variability of the gene expression among pools of microdissected colon tumors. These samples were analyzed using the Gene Discovery Array Human system (from genomesystems) that consists of two nylon membranes in a double spotted pattern at a density of 36,864 per spots per filter or 18,376 individual cDNA clones. Our preliminary results showed several differentially expressed clones between normal and tumor tissue analyzed. Our results were compared by software analysis to the patterns was found by Alon et al., 1999 using affymetrix oligonucleotide array complementary to more than 6,500 human genes tumor. When we compared our data against the results published by Alon et al, 1999 using UNIX program, 443 genes present differential expression in both analyses. Two hundred eighty were upregulate genes and 163 genes were downregulate. The majority of these genes are defined only by ESTs. Supported by: FAPESP.

Characterization of adjacent breast tumors using cDNA microarrays. *M.A Unger¹, M. Rishi², V. Clemmer³, B.L Weber¹*. 1) Department of Medicine, University of Pennsylvania, Philadelphia, PA; 2) Department of Pathology, St. Francis Hospital, Wilmington, DE; 3) Department of Surgery, St. Francis Hospital, Wilmington, DE.

The clinical assessment of a patient with multiple adjacent breast tumors or an in-breast recurrence can be difficult with respect to treatment. Currently used clinical methods of tumor analysis often can not determine if the tumors have arisen from a single cancerous cell or whether the tumors have developed from distinct clones. This information may be critical for determining a treatment strategy. The recent development and commercialization of cDNA microarray technology provides a promising means by which these uncertainties can be addressed by comparing the gene expression patterns between the tumors. We have tested the capabilities of commercially available cDNA microarrays to compare the gene expression patterns of two adjacent tumors excised from the right breast of an 87 year-old woman diagnosed with invasive ductal carcinoma (IDC). Total RNA was isolated from the tumors using Trizol reagent (Life Technologies) and arrayed in duplicate on Human Genome U95A Gene ChipTM (Affymetrix, Santa Clara, CA) containing 12,000 known genes. The data generated from the microarray experiments are being analyzed using Affymetrix software. The following characteristics are being considered with the gene expression patterns of the tumors: 1) the percentage of genes with a different expression levels between duplicate microarray experiments in a tumor, 2) the percentage of genes with different expression levels between the two adjacent tumors and the types of genes that are differentially expressed. This work provides an example of the potential of microarray experiments for tumor characterization and the potential for this technology to help guide clinical treatment.

SAGEmap: A gene expression resource for the Cancer Genome Anatomy Project. *G.J. Riggins¹, W.T. Loging¹, A. Lal¹, I. Siu¹, C. Boon¹, C. Turner¹, R.L. Strausberg²*. 1) Dept. of Pathology, Duke Univ Medical Ctr, Durham, NC; 2) Cancer Genome Anatomy Project, Office of the Director, National Cancer Institute, Bethesda, MD.

The Cancer Genome Anatomy Project (CGAP) has created an on-line system, SAGEmap, for the study of gene expression in tumors, normal tissues, and model systems (<http://www.ncbi.nlm.nih.gov/SAGE/>). This public resource is based on Serial Analysis of Gene Expression (SAGE), which is used to archive the numbers of expressed transcripts in each cell population. To date 2.4 million transcripts have been assayed from 54 different mRNA samples. In addition to being able to ascertain the expression levels in malignant and normal tissues, experimental systems are being used to locate candidate genes involved in basic oncogenic functions. SAGEmap is designed to help locate the downstream targets of certain oncogenes, genes induced by hypoxia, tumor antigens, genes conferring resistance to chemotherapy, genes involved in telomere maintenance and as well as other genes with a potential role in tumor formation. As a demonstration of this approach, results on the retrieval and functional analysis of one group of genes involved in glioblastoma will be presented.

The minimal region of CLN3 necessary for anti-apoptotic function resides at the carboxyl end. *R.N. Boustany^{1,2}, D.N Persaud^{1,2}, S. Dhar¹.* 1) Div Pediatric Neurology, Duke Univ Medical Ctr, Durham, NC; 2) University Program in Genetics, Duke University Medical Center, Durham, NC.

Juvenile neuronal ceroid lipofuscinosis is an autosomal recessive disease. Afflicted individuals manifest the disease as the progressive loss of vision, seizures, psychomotor disturbances and cognitive decline. The disease is caused by mutations occurring in the CLN3 gene. CLN3 consists of 15 exons and is approximately 1700 base pairs in length. The translated protein is 438 amino acids and 48 KDa. The most commonly observed mutation, affecting 80% of cases, involves a deletion of nucleotides 461-677 of the cDNA, which originates from a 1.02Kb deletion in the genomic DNA. This deletion falls within exons 7 and 8. The resulting protein is truncated and is 24 KDa. It has been shown that CLN3 is anti-apoptotic and acts by modulating ceramide generation upstream. In this study, deletion mutants were systematically generated from the 3' carboxy terminal of the gene and cloned into the pGEM (+7f) vector. Lymphoblast cell lines deficient in CLN3 were then transformed with these deletion constructs. Cell growth was then determined by thymidine incorporation and trypan blue cell counting. Restoration of the $ΔY_m$, or the mitochondrial membrane potential, of the transfectants was then assessed following treatment with etoposide using JC-1 staining. We show that exons 11-15 of the CLN3 gene provide transformed JNCL cells with protection from etoposide-induced apoptosis, and are also responsible for the correction of cell growth deficiencies. The goal of this project is to determine the motif or motifs present within this region that impart anti-apoptotic activity to CLN3. This can lead to effective drug screening for agents that can halt neurodegeneration.

Neurofibromatosis 2 (NF2) and malignant mesothelioma. *L.H. Pitts¹, R.K. Jackler^{1,2}, D. Altomare³, J.R. Testa³, M.E. Baser⁴*. 1) Department of Neurological Surgery, UC San Francisco, San Francisco, U.S.A; 2) Department of Otolaryngology, UC San Francisco, San Francisco, U.S.A; 3) Human Genetics Program, Fox Chase Cancer Center, Philadelphia, U.S.A; 4) Los Angeles, U.S.A.

NF2 tumors are caused by inactivating mutations of the *NF2* tumor suppressor gene, and somatic *NF2* mutations occur in a high proportion of malignant mesothelioma cells lines and primary tumors, but mesothelioma is not a characteristic feature of NF2. We describe a patient with NF2 and mesothelioma. Beginning at age 22, he was diagnosed with bilateral vestibular schwannomas, presenile lens opacities, and numerous spinal tumors. At age 40, he was diagnosed with a malignant peritoneal mesothelioma and he died shortly thereafter; he had worked as an automotive and jet mechanic since age 15, and the causal exposure probably occurred while working on brake systems or from reworking asbestos-containing parts. This case is consistent with Knudson's observation as to why mutations in the same tumor suppressor gene can cause different tumor types that do not each occur as part of hereditary cancer syndromes (PNAS 1995;92:10819-20). Tumors that are characteristic of hereditary cancer syndromes occur in tissues whose stem cells proliferate in utero or in adolescence (e.g., neuroectoderm-derived NF2 nervous system tumors), but proliferation of frequently-renewed tissues (e.g., malignant transformation of the mesoderm-derived peritoneum) requires an additional pathological condition. Molecular studies will include (1) *NF2* and LOH analyses, (2) comparative genomic hybridization to compare the patterns of genetic imbalances in benign and malignant tumors, and (3) molecular and immunohistochemical assays of changes in other tumor suppressor genes that often occur in mesothelioma (e.g., *p16/CDKN2A*).

Predictors of vestibular schwannoma growth in neurofibromatosis 2 (NF2). *D.M. Parry*¹, *E. Makariou*², *M.E. Baser*³. 1) Genetic Epidemiology Branch, National Cancer Institute, Bethesda, MD; 2) Department of Radiology, Georgetown University School of Medicine, Washington, D.C; 3) Los Angeles, CA.

We previously reported that NF2 vestibular schwannoma (VS) growth rates varied inversely with age (Am J Hum Genet 1998;63:[4 Suppl]A63), but others have found a direct relationship (Laryngoscope 1996;106:694-699). To resolve this discrepancy, we re-analyzed data from the U.S. National Institutes of Health longitudinal study of NF2, on whose patients the Laryngoscope study was also based. VS growth rates were expressed as tumor doubling times (TDTs, in years) to account for differences in baseline VS volumes. The median length of follow-up in 15 patients was five years. Fourteen patients had identified constitutional *NF2* mutations (five nonsense or frameshift mutations, six splice-site mutations, three in-frame deletions). The median TDT in patients ≤ 30 years old was 15 years, compared to 35 years in patients > 30 years old, indicating a higher VS growth rate in younger patients. Median TDTs were similar in patients with nonsense or frameshift mutations (31 years), splice-site mutations (34 years), and in-frame deletions (37 years). TDTs were inversely associated with the number of intracranial meningiomas ($r^2 = .35$, $P = .02$). This finding extends other studies that have found that the number of meningiomas is an important feature of NF2 disease severity and is an independent predictor of survival in NF2.

Human hepatocellular carcinoma is characterized by a highly consistent pattern of genomic imbalances, including frequent loss of 16q23.1-24.1. *B.R. Balsara¹, J. Pei¹, A. De Rienzo¹, D. Simon², A. Tosolini¹, Y.Y. Lu³, F. Shen⁴, X. Fan⁴, W.Y. Lin⁴, K.H. Buetow⁵, W.T. London¹, J.R. Testa¹.* 1) Div. of Population Sciences, Fox Chase Cancer Center, Philadelphia, PA; 2) MCP-Hahnemann School of Medicine, Philadelphia, PA; 3) Department of Biochemistry and Molecular Biology, Beijing Institute for Cancer Research, Beijing, P.R. China; 4) Shanghai Medical University, Haimen City Cancer Prevention Institute, Shanghai, P.R. China; 5) Laboratory of Population Genetics, National Cancer Institute, Bethesda, MD.

Comparative genomic hybridization (CGH) analysis was used to identify chromosomal imbalances in 52 human primary hepatocellular carcinomas (HCCs). The most prominent changes were gains of part or all of chromosome arms 8q (83% of cases) and 1q (73%) and loss of 16q (63%). Other commonly overrepresented sites were 5p, 7q, and Xq. Recurrent sites of DNA sequence amplification included 8q23-24 (5 cases) and 11q13-14 (4 cases). Other frequently underrepresented sites were 4q, 8p, 16p, and 17p. Taken collectively, these findings and data from other CGH studies of HCCs define a subset of chromosome segments that are consistently over- or underrepresented and highlight sites of putative oncogenes and tumor suppressor genes, respectively, involved in hepatocellular oncogenesis. Loss of heterozygosity analysis with a panel of polymorphic microsatellite markers distributed along 16q defined a minimal region of chromosomal loss at 16q23.1-24.1, suggesting that this region harbors a tumor suppressor gene whose loss/inactivation may contribute to the pathogenesis of many HCCs.

Genotoxic effect of breast cancer patient's serum and plasma on normal blood leucocytes. *r. narayanappa*^{1,2}, *A. Kumar*¹, *Y.R. Ahuja*². 1) MRDG, Indian Institute of Science, Bangalore 560012, India; 2) Genetics unit, Bhagawan Mahavir Medical Research Center, 10-1-1, Mahavir marg, Hyderabad 500004, India.

Most human cancers are associated with genetic instability. Our previous studies have shown increased level of DNA damage in the peripheral blood leucocytes of cervix and breast cancer patients as compared to the controls. The presence of increased DNA damage in the leucocytes in these cancer patients which is not the target tissue is rather unexpected. According to Werkeimester et al (1980), some genotoxic agents are being released by the tumor tissue into the blood stream which might be the cause for this increased DNA damage observed in the leucocytes. To investigate the genotoxic activity of the cancer patient's blood, the genotoxic activity of the sera and plasma from breast cancer patients were evaluated comet assay. Comet assay is simple, sensitive technique used to quantitate DNA single strand breaks, double strand breaks and alkali labile sites at individual cell level. Serum and plasma were isolated from 10 untreated breast cancer patients. Normal individual's lymphocytes were treated with breast cancer patient's serum and plasma. DNA damage was evaluated by comet assay after 1hr and 2hr treatment. A significant increase in the level of mean comet tail length was observed in the normal lymphocytes treated with the breast cancer patient's serum as compared to the untreated normal lymphocytes. Similarly, a significant increase in the level of mean comet tail length was observed in the normal lymphocytes treated with the breast cancer patient's plasma as compared to untreated normal lymphocytes. This increased DNA damage in the normal lymphocytes could be due to the presence of some genotoxic factors in the serum and the plasma of the breast cancer patients. This is a preliminary study carried out to check out on the genotoxic effect of breast cancer patient's blood. Further detailed study need to be carried out to come to reliable conclusion.

HPV16 Integrations in Cervical Tumors Preferentially Occur within the Common Fragile Sites and Cluster at Specific Sites. *E.C. Thorland¹, S.L. Myers², B.S. Gostout³, D.I Smith⁴*. 1) Biochem & Molec Biol, Mayo Clinic, Rochester, MN; 2) Tumor Biology Program, Mayo Clinic, Rochester, MN; 3) Obstetrics and Gynecology, Mayo Clinic, Rochester, MN; 4) Division of Experimental Pathology, Mayo Clinic and Foundation, Rochester, MN.

The development of cervical cancer is highly associated with human papillomavirus (HPV) infection, and HPV integration is temporally associated with the acquisition of the malignant phenotype. A relationship between the sites of HPV16 integration and the positions of the common fragile sites (CFS) has been observed at the cytogenetic level. We explored this relationship at the molecular level. We utilized a PCR-based method to amplify the junctions between HPV sequences and the flanking genomic sequences in cervical tumor specimens. Primers based on the flanking sequences were then used to screen BAC libraries to isolate genomic clones at each integration site. These were then used as FISH probes against aphidicolin-induced metaphase chromosomes. We isolated genomic sequences flanking HPV integration sites from 20 HPV16-positive cervical tumors. Many of these integrations occur within the CFS verifying our hypothesis. The integrations from the 20 cervical tumors occurred on many chromosomes, but we have observed specific clustering of the integrations at 17q23 (the site of FRA17B), and 13q21-31 (the sites of FRA13B and FRA13C). Three integrations occurred within a 2 Mb interval on 17q23 surrounding FRA17B. Two integrations occurred within a less than 200 Kb interval on chromosome 13. Sequence analysis demonstrated that the chromosome 13 integrations occurred within the same interval as the integration previously cloned from the cervical tumor cell line, SiHa. It has been proposed that HPV integration events in cervical tumors may disrupt cellular genes at the integration sites. The observed clustering of integrations suggest that genes important in the initiation or progression of cervical cancer may be specifically disrupted by these integrations. We are currently characterizing these two regions to determine if genes contained within them have a role in cervical carcinogenesis.

Loss of heterozygosity correlates with tumor and host response parameters in squamous cell carcinoma of the head and neck (SCCHN). *D.L. Van Dyke¹, J.A. Gomez², A.T. Savera², B. Rybicki³, M.S. Benninger⁴, R.J. Zarbo².* 1) Departments of Medical Genetics; 2) Pathology; 3) Biostatistics & Research Epidemiology, and; 4) Otolaryngology, Henry Ford Hospital, Detroit, MI.

Little is known of genotypic associations with histologic phenotypic features in SCCHN. Therefore, we evaluated the correlation between loss of heterozygosity (LOH) at several chromosomal loci and objective histopathologic characteristics. A series of 107 SCCHN were histologically typed and scored in a 4 tier scheme for histologic parameters (keratinization, nuclear grade, mitoses per high power field) and host interface characteristics (inflammation, desmoplasia, and pattern of invasion). PCR was performed on DNA isolated from paraffin tumor blocks and normal tissue for LOH at eight MSRP loci (3p13, 3p21, 5q12, 8p22, 9p21, 10p13, 18q13, 21q11). Dichotomous factors were compared by chi square test and ordinal factors by Kendall's tau, to test for associations with LOH. Of 107 patients with pathologic data, paired normal and tumor LOH data are available for 69. Tumors were classified as conventional (83%), non-keratinizing (8%), papillary squamous (3%), basaloid squamous (1%), adenosquamous (1%), conventional/non-keratinizing (1%) and conventional/ adenosquamous (1%). Statistically significant associations were noted for a poorer histologic grade and LOH at 3p21 ($p=0.05$), and the data were suggestive of an association between grade and LOH at 3p13 and 5q12 ($p=0.11$ for both). Decreasing inflammatory response was significantly associated with LOH at 3p21, 3p13, 5q12, and 9p21 ($p=0.004$, $p=0.05$, $p=0.06$, and $p=0.04$, respectively). Increased numbers of mitoses was significantly associated with LOH at 18q13 ($p=0.04$). These findings suggest that LOH at certain loci on chromosomes 3, 5, 9, and 18 correlate with more aggressive histologic features. The present findings suggest that there are morphologic correlates of our previously reported findings of significantly poorer survival for patients whose tumors exhibit evidence of genetic loss at loci on chromosome 3, 5, and 18 (Pearlstein et al. *Genes Chrom Cancer* 21:333-339,1998, and in abstracts elsewhere.).

Hypermethylation in the 5' region of the GSTP1 gene is a potential diagnostic index for prostate carcinoma. D.C.

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Background: Prostate cancer has been ranked as one of the top ten male cancer syndromes in Taiwan recently. A sensitive and specific biomarker is desired for diagnosis of prostate cancer. It was found that extensive methylation occurred in the 5' CpG island flanking the p-class glutathione S-transferase (GSTP1) gene in prostate cancer cells but not in normal cells. **Objective:** the purpose of this study is to investigate whether this genomic alteration could be used as a diagnostic index for prostate cancer. **Study design:** Genomic DNA was extracted from paired tissue and blood samples from prostate cancer patients. After methylation sensitive restriction enzyme Hpa II treatment, nested PCR was performed to amplify the 5' region flanking the GSTP1 gene. PCR amplicon was then analyzed qualitatively. **Results:** the preliminary data showed that of the 6 tissue samples analyzed, 6 of 6 (100 %) showed positive band patterns indicating hypermethylation in this region. However, the same band patterns were not observed when corresponding blood samples were analyzed. **Conclusions:** Hypermethylation of CpG islands in the promoter region of the GSTP1 gene appeared in high frequency in prostate cancer cells. It suggested potential application of molecular diagnosis of prostate cancer with this marker. Next, more prostate cancer samples will be analyzed to determine the detecting ability of this novel biomarker.

Identification and characterisation of a founder mutation in the Belgian breast/ovarian cancer population. K.

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In order to identify the mutation spectrum of the *BRCA1/2* genes in the Belgian patient population, we perform mutation analysis for the complete coding sequence of both genes in Belgian breast and ovarian cancer patients. We finished the analysis in 73 families fulfilling our inclusion criteria (Claes et al, 1999) and in 28 patients diagnosed with breast and/or ovarian cancer before age 35. In about 30% of the families a mutation in *BRCA1* or *BRCA2* was identified. 75% of the mutations were detected in *BRCA1*. In none of the sporadic patients a mutation was found. Some mutations are novel and never reported in other populations.

In 7 unrelated families the same splice site mutation in *BRCA1* was identified: IVS5+3A>G. Haplotype analysis suggests that they share a common ancestor. This mutation was reported by Peelen et al (1997) in another Belgian family, but has not yet been identified in other populations.

All our 7 families harbouring the mutation IVS5+3A>G represent a severe phenotype: patients are often diagnosed with breast cancer at rather young age (Dx<40) and ovarian cancer is present in 4/7 families.

At the mRNA level *BRCA1* IVS5+3A>G inactivates the splice donor site of exon 5 and activates a cryptic donor (ctttAT/GTaaga) present in exon 5 leading to out of frame skipping of the last 22 nucleotides of exon 5 and a premature stop codon. Interestingly this splice variant was observed also in the lymphocytes of patients without this splice site mutation, albeit typically at low level. Additional tissues, tumors and tumor cell lines are currently investigated in order to further characterise a possible functional significance of this splice variant.

Nibrin, the molecule mutated in Nijmegen breakage syndrome, is required for correct nuclear localization and focus formation by a DNA repair protein complex. A. Desai-Mehta, K. Cerosaletti, P. Concannon. Virginia Mason Research Center and University of Washington School of Medicine, Seattle, WA.

Patients with the autosomal recessive disorder Nijmegen breakage syndrome (NBS) display microcephaly, growth retardation, chromosomal instability, hypersensitivity to ionizing radiation, immunodeficiency and an increased incidence of malignancies, primarily of lymphoid origin. NBS results from truncating mutations in the *NBS1* gene that encodes the protein nibrin. In normal fibroblasts, nibrin is part of a nuclear multiprotein complex that contains the DNA repair proteins Mre11 and Rad50. Upon irradiation, the nibrin/Mre11/Rad50 complex redistributes to form distinct nuclear foci that are thought to be sites of DNA repair. In cell lines from NBS patients, nibrin is truncated or absent, Mre11/Rad50 proteins lose their nuclear localization and foci fail to form upon irradiation. This suggests that the sequences in the carboxy-terminal half of nibrin are necessary for the maintenance of this protein complex. Here, we report the mapping of the sites of interaction on nibrin and Mre11, respectively, using the yeast two-hybrid system and expression of epitope-tagged constructs in normal and NBS fibroblasts. Sequences in the carboxy-terminal 101 amino acids of nibrin interacted with the amino-terminal region of Mre11. Retroviral expression vectors that produced either wild-type nibrin or a mutant form lacking the Mre11 binding site were constructed. Introduction of the wild-type nibrin expression vector into NBS cells fully complemented the phenotype including nuclear localization of complexes of nibrin, Mre11, and Rad50, formation of nuclear foci, and survival after irradiation. In contrast, the mutant nibrin displayed nuclear localization but failed to interact with or relocalize Mre11 or Rad50. Upon irradiation, the mutant nibrin formed the characteristic nuclear foci, but survival of the cells was only slightly improved. These results suggest that nibrin is necessary and sufficient to direct nuclear localization and focus formation either alone, or in a complex with Mre11 and Rad50. However, direct interaction with Mre11 is required for normal cellular survival post-irradiation.

Screening individuals at high risk for breast or ovarian cancer by BRCA 1/2 mutation analysis: Experience from a national, comprehensive educational program. A.T. Bombard¹, A.W. Cohen², T.S. Frank³, A. Deffenbaugh³. 1) Aetna US Healthcare; San Ramon, CA/Albert Einstein College of Medicine; Bronx, NY; 2) Aetna US Healthcare; Blue Bell, PA/Jefferson Medical College; Philadelphia, PA; 3) Myriad Genetics Laboratory; Salt Lake City, UT.

Introduction: Advances in molecular susceptibility testing provide an opportunity to effect true preventive health care by identifying women at greatest risk for familial breast/ovarian cancer *before* these cancers are clinically detectable. Once identified, at-risk individuals may utilize a variety of preventive and therapeutic options that are predicted to improve health and increase longevity. Aetna U.S. Healthcare, working with Myriad Genetic Laboratories, has developed a national, comprehensive program to educate 21,000,000 Americans and their providers about heritable breast and ovarian cancers. The project has included distribution of informational brochures to ~120,000 providers; AMA-developed CME for physicians; a simple, "Pre-Authorization" form for pre-approval coverage of testing; coverage of both medical and surgical therapies; and confidentiality (all test results are directed to the provider only). Initial AUSHC Program vs Overall Myriad Results: 1. Deleterious mutations were identified in 51/277 women (18 %, vs 16% overall); 2. Among women with no personal history of breast cancer < 50, or ovarian cancer at any age, deleterious mutations were identified in 22/277 women (8%, vs 9% overall); 3. Among women with either diagnosis, mutations were identified in 97/277 women (35%, vs 23% overall). Summary: These results show that a comprehensive, national genetics program focusing on breast and ovarian cancer risk education, screening those at greatest risk using BRCA sequencing, and coverage of current medical and/or surgical therapies can be successfully implemented. Moreover, results of this broad-based program within the general clinical provider community has achieved results comparable to those found in academic medical centers - the principal sample source in the Myriad Genetics overall laboratory experience. Specific program details and the results of the screening experience will be presented.

The role of DNA mismatch repair defects in haematological malignancy. *S. Andrew¹, M. Campbell¹, F. Jirik², R. Coupland³.* 1) Dept Medical Genetics, Univ Alberta, Edmonton, Alberta, Canada; 2) Centre for Molecular Medicine and Therapeutics, Vancouver, BC Canada; 3) Laboratory Medicine and Pathology, Cross Cancer Institute, Univ Alberta, Edmonton, Alberta, Canada.

Germline mutations in the DNA mismatch repair (MMR) genes have been implicated in the genesis and progression of human malignancy as individuals develop hereditary non-polyposis colorectal cancer. Microsatellite instability (MSI), which can be indicative of loss of MMR, is characteristic of a variable but considerable percentage of many human malignancies, suggesting loss of MMR is an important tumorigenesis mechanism. The role of MMR in haematological malignancy has not been widely studied, however, the finding that mice homozygous for a lack of MMR activity also demonstrate a tumour-prone phenotype, especially the development of thymic lymphomas, suggested MMR may also be important in human haematological malignancy. Recent reports of a few rare children with two inherited MMR mutations that develop leukemias and lymphomas also suggested a link between loss of MMR and human haematological cancers. We chose to study loss of MMR in human T-cell lymphoblastic lymphomas (LBL), the equivalent of the murine T cell lymphomas that develop in Msh2 deficient mice. Two of ten (20%) of T-cell LBLs tested to date demonstrated a MSI+ phenotype, and loss of MSH2 by immunohistochemistry. The inactivating mutations within MSH2 are currently being sought. These MSI+ tumours can now be used to screen candidate genes postulated to be likely non-random targets for mutation in the absence of MMR. Several such genes have been identified in HNPCC or MSI+ colorectal, endometrial tumours etc. We will report on screening of TGFRII, IGFRII, BAX, PTEN, and other likely candidates for mutations in human T-LBLs as well as within the murine Msh2^{-/-} thymic lymphoma DNA. Identification of genes involved in the proportion of lymphomas that develop along the MSI+ pathway will offer a new understanding towards the molecular nature of lymphomagenesis.

Detection of 11 germline inactivating *TP53* mutations and absence of *TP63* and *hCHK2* mutations in 17 French families with Li-Fraumeni or Li-Fraumeni-like syndrome. G. Bougeard¹, J.M. Limacher², C. Martin¹, F.

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The Li-Fraumeni syndrome (LFS), affecting children and young adults, represents one of the most devastating genetic predispositions to cancers and is characterized by a wide spectrum of early-onset malignancies including bone and soft-tissue sarcomas, brain tumours, adrenocortical tumours and premenopausal breast. Germline *TP53* mutations have been detected in most of the families with LFS. Recently germline mutations of *hCHK2*, encoding a DNA damage checkpoint kinase, were identified in LFS families, indicating the genetic heterogeneity of this syndrome. We analyzed 17 French families, including 4 families fulfilling the complete criteria for LFS, and 13 the criteria for the Li-Fraumeni-like syndrome (LFL). The *TP53* functional assay in yeast and sequencing analysis of exons 2 to 11 led to the identification of 11 inactivating germline mutations in 4/4 LFS and 7/13 LFL families, including 6 unreported germline mutations. In the families without *TP53* germline mutation, we analyzed by a new functional assay and sequencing analysis the *TP53* homolog, *TP63*, which isotype γ is able to transactivate the *TP53* target genes, and we detected no alteration. Sequencing analysis of the entire coding region of the *hCHK2* gene within these families also identified no alteration. This study shows that the functional assay is an efficient method to detect germline *TP53* mutation since, in this series, it detected all the germline mutations, confirms that most of the LFS/LFL cases result from germline inactivation of *TP53* and indicates the involvement of other genes in the syndrome.

Heritable versus sporadic paraganglioma: High proportion of heritable cases and high predictability of carotid body tumor diagnosis for heritable cases. *C.M. Drovdic¹, E.N. Myers^{2,3}, J.A. Peters^{1,2}, B.E. Baysal^{1,4}, D.E.*

Brackmann⁵, W.H. Slattery III⁵, D.B. Kameron³, B.E. Hirsch³, R.L. Carrau³, J.T. Johnson³, C.H. Snyderman³, W.S. Rubinstein^{1,2}. 1) Dept Human Genetics, Univ Pittsburgh Graduate School of Public Health, PA; 2) Univ Pittsburgh Cancer Inst, PA; 3) Univ Pittsburgh Eye and Ear Inst, PA; 4) Univ Pittsburgh Dept Psychiatry, PA; 5) House Ear Inst, Los Angeles, CA.

Paragangliomas (PGL) are mostly benign tumors of the head and neck that affect about 1/30,000 individuals. Reportedly, 7-50% are hereditary. A major gene responsible for these tumors, PGL1, on chromosome 11q23, was identified as succinate-ubiquinone-oxidoreductase subunit D (*SDHD*). There is evidence for a PGL2 locus on chromosome 11q13 and PGL3 locus at an unknown location. Hereditary predisposition to PGL due to PGL1 or PGL2 is inherited in an autosomal dominant fashion with maternal genomic imprinting. PGL3 does not show evidence of imprinting. Identifying heritable PGL cases reveals at-risk relatives and has medical management/genetic counseling implications. We wanted to determine the heritable proportion of PGL by detailing and classifying subjects' medical and family histories. Subjects were patients diagnosed with PGL irrespective of family history. We defined heritable as a case with a positive family history and/or bilateral or multifocal PGL. Of 60 subjects, 21 were classified as heritable. Our data suggest that approximately 35% of patients that present to an otolaryngologist with PGL have a hereditary predisposition recognizable by clinical criteria. Subjects diagnosed with a non-bilateral carotid body tumor (CBT) upon first diagnosis were 5.8 times more likely to have heritable PGL than those diagnosed with PGLs at other anatomical locations in the head and neck. Heritable subjects had non-statistically significant younger average ages of onset than non-heritable subjects, reflecting a trend in the literature. Using clinical criteria, including family history, bilaterality and multifocality, we estimate the heritability of PGL to be 35%. Our study suggests that diagnosis of CBT may also be used as a clinical criterion of heritability.

Multiple Primary Melanoma, Family History, and Germline Mutations in CDKN2A. A. Ganguly¹, S.N Palmer¹, M. Young², R. Holmes², L.M Schuchter², D. Guerry², M.A Blackwood². 1) Department of Genetics, Univ Pennsylvania, Philadelphia, PA; 2) University of Pennsylvania Cancer Center, Philadelphia, PA.

We examined family history and germline mutations in CDKN2A gene in a cohort of 75 patients with invasive multiple primary melanomas (MPM). Family histories were collected by a genetic counselor and cancer incidences were documented by pathology report. A 3-generation pedigree was assembled for each study subject that enabled the tracking of melanoma and other cancer histories in affected families. We used polymerase chain reaction and direct DNA sequencing of the 3 coding exons of CDKN2A from genomic DNA isolated from peripheral blood lymphocytes and to identify germline mutations in the study subjects. Pedigree analysis revealed, that a small subset, 2/75(3%), of MPM subjects had a family history with the proband and more than 2 additional members affected with melanoma. Another subset, 5/75(6.6%), had at least 1 additional first degree relative with melanoma while the majority had limited family history of melanoma. Thus multiple primary melanoma commonly present without any evident genetic predisposition. Six subjects (8%) were found to have mutations in coding sequences of CDKN2A. These mutations include 4 missense mutations, and 2 deletion/insertion mutations. Of the 4 missense mutations, G23C, V28G, M53I, and G101W, the latter two have been reported in familial melanoma. The 279del14 mutation leads to a truncated protein and Ins24 gives rise to an in frame duplication of 8 amino acids at the 5'-end of the gene. Five individuals are positive for the A148T polymorphism and 12 were positive for either one of the 3-UTR polymorphisms(C540G and C580T). Family history of melanoma was documented in 3 out of the 6 CDKN2A mutation carriers. We conclude that the prevalence of CDKN2A mutations in this unselected cohort of patients with multiple primary melanomas is lower than 15% reported by Monzon et al(NEJM338: 879,1998). The presence of germline CDKN2A mutations in individuals with no family history of melanoma suggest the occurrence of de novo germline mutations in this cancer predisposing gene and/or low penetrance for the same mutations.

Anticipation in Familial Breast Cancer. *E. Dagan*¹, *R. Gershoni-Baruch*^{1,2}. 1) Institute of Human Genetics, Rambam Medical Ctr, Haifa, Israel; 2) Bruce Rappaport Faculty of Medicine, Technion - Institute of Technology, Haifa, Israel.

To test for evidence of anticipation in familial breast cancer we collected clinical, socio-demographic and genetic data on mother/daughter pairs with breast cancer. Eighty four Ashkenazi women with breast cancer whose mothers were diagnosed with breast cancer were genotyped in respect to their being carriers of an Ashkenazi founder mutation in either BRCA1 (185delAG, 5382insC) or BRCA2 (6174delT). The phenomenon of anticipation was then related to the presence of founder mutations in BRCA1/2. Mean age of onset was higher in the maternal generation (55.6 +/-14.3 years) than in the daughters generation (48 +/- 9.5 years) ($t=4.1$; $p<0.001$). Twenty seven mutation carriers were identified, 17 carried a BRCA1 mutation and 10 carried the 6174delT mutation in BRCA2. Among BRCA1 mutation carriers the mean age at diagnosis in the mothers (45.3 +/-13) did not differ from that reported in the daughters (42.4 +/- 9). Among BRCA2 mutation carriers and non-carriers the mean age at diagnosis in the daughters (41.4 +/- 7.2 and 50.7 +/- 8.8, respectively) was significantly younger than in the mothers (62.4 +/- 13 and 57.4 +/- 13.6, respectively) ($t=-5.5$; $p<0.001$ for BRCA2 carriers and $t=-3.7$; $p=0.001$ for non-BRCA1/2 carriers). In the mothers' generation carriers of BRCA1 mutations were diagnosed with breast cancer at a significantly earlier age than carriers of BRCA2 mutations and non-carriers. In the daughters' generation carriers of BRCA1 and BRCA2 mutations were diagnosed with breast cancer at a significantly earlier age than non-carriers. Our findings indicate that BRCA1 mutations predispose to breast cancer at an early age in both mothers and daughters, whereas mutations in BRCA2 are associated with significantly earlier age of onset in the second generation. This observation could be related to gene-environmental interactions causing anticipation in BRCA2 mutation carriers.

Point mutations in PTEN in primary carcinomas and uninvolved tissue of breast, colon, endometrium, kidney, and lung. *L. Gui*¹, *C.S. Ringelberg*², *M. Kaelbling*². 1) Pathology; 2) Preventive Medicine, U. Mississippi Medical Center, Jackson, MS.

As part of our ongoing mutation analysis of putative tumor suppressor genes, we identified DNA sequence alterations in the lipid and protein phosphatase gene PTEN/MMAC1/TEP1 (PTEN) in 62 tumor and uninvolved tissue panels of the same patients: 11 breast, 14 colon, 13 endometrium, 12 kidney, and 12 lung. Initial studies found 10q-LOH in many glioblastoma, breast, colon, lung, prostate, and digestive tract cell lines or tumors, while point mutations or small nucleic acid sequence changes were found only in the brain tumors. Subsequently, PTEN point mutations and LOH were also found frequently in endometrial carcinoma and, less often, in malignant melanoma; liver, kidney, and thyroid carcinoma. Germline PTEN mutations were associated with juvenile hamartomatous polyposis syndromes including Cowden disease, juvenile polyposis syndrome, and Bannayan-Riley-Ruvalcaba syndrome. Most PTEN alterations occurred in exons 5 and 7, while most exon 8 sequence changes appeared to be polymorphisms.

We analyzed PTEN alterations in exons 5, 7, and 8 by SSCP and sequenced bands that differed within or between panels. Definite mutations were found in seven panels and two exons. Exon 5 yielded two missense and one frameshift mutation: R130G occurred in three endometrial tumors and one matching uninvolved tissue, N107V was on the other allele of one of these tumors; 409delG was present in a kidney tumor. Exon 7 yielded one missense, one nonsense, and one frameshift mutation: D252G occurred in a lung tumor; R233X and 739delT in different endometrial tumors. No alterations were detected in exon 8. We are now sequencing additional panels because the exon transcript sizes (262-313 bp) are somewhat large for optimal SSCP analysis.

All tumor samples contained normal stroma while some uninvolved/constitutive tissue contained a tumor focus. Of the panels, 50 were obtained from the Cooperative Human Tissue Network and 12 were collected at our institution.

Outcomes from an enhanced cancer genetics education program for clinicians. *K.R. Blazer, D.J. MacDonald, S. Sand, M. Grant, J.N. Weitzel.* Clinical Cancer Genetics and Nursing Research and Education, City of Hope Cancer Ctr, Duarte, CA.

Advances in understanding the genetic basis for cancer has led to new technologies for hereditary cancer risk assessment. Identification of patients and families at high risk for inherited cancer depends upon increasing primary care physicians' knowledge of the fundamentals of genetics and risk assessment. A Cancer Genetics Education Program (CGEP) was developed to educate healthcare professionals about cancer genetics and inherited cancer risk. State-of-the-art information was delivered through regular hospital-based one-hour CME seminars, four full-day regional conferences and an educational internet website. Over 5,100 allied health professionals (oncologists, surgeons and primary care physicians 71%; nurses, genetic counselors and allied health professionals 29%) participated since 1997. We used innovative approaches to garner participation by community physicians in full-day conferences, including partnering with community health organizations and inviting physicians to present cases from their practice in a highly rated practical applications segment of the conference. The website (www.cityofhope.org/ccgp) received more than 1,000 queries and was also used for online registration. Scores from pre- and post-seminar questionnaires demonstrated significant improvement in cancer genetics knowledge (overall 40% average increase); increase in appropriate referrals to the *Cancer Screening & Prevention Program* by attendees reflected an impact on clinical practice. Baseline knowledge was greater among participants in full-day courses (56% correct answers), reflecting possible selection for clinicians interested in cancer genetics, compared to scores sampled from one-hour community hospital-based CME seminars (39%). Knowledge was increased in both settings (by 26% and 54%, respectively). CGEP experience to date suggests that both formats are necessary to reach a broad audience of primary care physicians, and that the full day courses may confer screening-level competence to a motivated subset. Funded in part by NCI-1R25CA75131; and project #MCHG-51, Maternal Child Health Bureau.

Gene Expression in Prostate Cancer; Microarray Analysis of Tumor Specimens and the LNCaP Prostate Tumor Model Series. *C.L.T. Clelland, D.M.P Morrow, C. Bancroft.* Dept Physiology & Biophysics, Mount Sinai School of Medicine, New York, NY.

Prostate cancer is the second largest cancer killer of men. In 1999, nearly 200,000 men were diagnosed with prostate cancer in the United States, with an estimated 37,000 cancer deaths in that year alone. During progression of the disease the prostate tumor loses its androgen sensitive state, becoming non-responsive to standard androgen ablation therapy. The LNCaP prostate tumor model series consists of lineage-related sublines that differ in growth abilities and recapitulate some of the major stages in the progression of this disease. For these reasons this model series has been utilized for prostate cancer research. The initial LNCaP cells have the ability to form tumors in non-castrated nude mice, but only upon co-injection of the MS bone marrow cell line. The second in the series, the C4 line, is androgen independent (and can form tumors in castrated nude mice), but the formation of tumors still requires bone cell injection. The C42 line however, has lost both androgen and bone cell dependence, while the C42B line represents the last stage of the series, with the ability to metastasize to bone.

We have used microarray analysis to identify genes differentially expressed among the individual stages of the LNCaP series. Total RNA was extracted from each cell line, in duplicate, and labeled cDNA was produced from each sample, using the NEN MicroMaxTM reagents. cDNAs were then hybridized, in duplicate, to a MicroMaxTM microarray slide, which contains 2400 human cDNAs obtained from 50 tissue sources, each characterized functionally on the basis of Prosite motif search results. We have employed this labeling and microarray system to repeat experiments using RNA extracted from histologically graded and Gleason scored tumor prostatectomy specimens. Following scanning and extrapolation of fluorescent signal data, computer algorithms were employed to compare expression patterns between the LNCaP series and the tumor specimens. We discuss the use of the LNCaP series in the analysis of expression patterns of prostate cancer.

The impact of genetic counseling/testing for breast and ovarian cancer in a free and anonymous community

program. *T. Bardakjian*^{1,2}, *N. Shields*², *P. Gilman*², *E. Olander*^{1,2}, *S. Dickinson*^{1,2}, *S. Atassi*², *J. Mushlin*², *A. Schneider*¹, *M. Dabrow*^{3,2}. 1) Albert Einstein Med Ctr, Philadelphia, PA; 2) Lankenau Hospital, Wynnewood, PA; 3) Jefferson Med College, Philadelphia, PA.

A program was established in 1998 to evaluate the impact of free and anonymous genetic counseling/testing for breast/ovarian cancer provided in a suburban medical center. The primary aim was to assess a woman's risk for developing these cancers and to offer those at high risk genetic counseling and testing, as appropriate. We proposed that this service would impact cancer screening and prevention methods in the study group. Women were asked to complete a brochure about their family history and personal risk factors. Based on this information, women were stratified into 3 groups: average, somewhat higher than average, or high risk. Genetic counseling was offered to all those at high risk. There were 55 women/families in this category, 33 of whom, completed the genetic counseling process. Thirty of the 33 were offered testing, and 28 (93.3%) of these underwent testing. Six individuals were found to carry a BRCA mutation. Follow-up telephone questionnaires at 12 months after disclosure evaluated screening and prevention practices and overall satisfaction with genetic testing. Six of 28 (21.4%) women tested initiated monthly self-breast exam after participation in the program, irrespective of their gene test result. All 27 women tested stated they were "mostly satisfied" with their decision to be tested. Twenty-five of 26 felt that knowing their results did not interfere with their daily activities while 1/26 felt that her positive result "somewhat interfered" with her daily living. In conclusion, risk assessment, genetic counseling and testing can have a positive impact on a woman's breast cancer surveillance, regardless of test results and without causing excess anxiety. The availability of free and anonymous genetic testing has resulted in a remarkably high acceptance rate of genetic testing. This demonstrates that gene testing in conjunction with appropriate genetic counseling and education is an important tool for women at high risk for breast/ovarian cancer.

Breast, ovarian and other cancer penetrance estimates for BRCA1 carriers identified in a cancer risk evaluation program. *M.S. Brose¹, T.R. Rebbeck², K.L. Nathanson¹, B.L. Weber.¹* 1) Dept. of Medicine, Div. of Hematology and Oncology, Hospital of the University of Pennsylvania, Philadelphia, PA; 2) Center for Clinical Epidemiology and Biostatistics, The Univ. of Penn., Philadelphia, PA.

Increasing numbers of BRCA1 mutation carriers are being identified in cancer risk evaluation programs and estimates of cancer penetrance from these clinics would be useful, as linkage families may overestimate, and population-based series may underestimate, risk. Previous lifetime penetrance estimates range from 36% to 87% for breast cancer, and 16% to 60% for ovarian cancer. We have extended our initial studies on the pedigrees of BRCA1 families identified in our cancer risk evaluation clinic to determine the risk of breast, ovarian, and other cancers in both BRCA1 positive tested individuals and obligate carriers. In 191 female BRCA1 mutation carriers the lifetime breast cancer risk was estimated at 64%, less than reported in families used for linkage analysis but much greater than estimates derived from a population selected for Tay-Sachs screening. To evaluate the bias toward testing affected women, we carried out additional analyses for 70 obligate carriers and found a lifetime penetrance of 60%. The lifetime risk of ovarian cancer in the BRCA1 tested carriers was 23% and in obligate carriers was 33%, as compared to 40-60% percent in linkage studies, and 16.5% for an Ashkenazi Jewish volunteer-based study. We have also investigated the incidence of other cancers including colon, prostate, pancreatic, and lung cancer and found that these cancers are found in greater proportion of our obligate carriers than in the tested individuals. This may be due to differences in the ages of the obligate and tested carriers or the natural bias toward breast cancer in our clinic population. Additional analyses are underway. We conclude that the risk of breast and ovarian and other cancers for BRCA1 mutation carriers identified in high risk programs is significantly different from some previous estimates. Penetrance estimates of all cancers derived directly from the population of influence may better represent the risk faced by the majority of carriers identified.

The presence of a germ-line *BRCA* mutation is an independent poor prognostic marker in breast cancer: a two-center historical cohort study. *W.D. Foulkes¹, J.M. Satagopan², P.O. Chappuis¹, N. Wong¹, L.R. Bégin¹, J. Boyd², K. Offit², M.E. Robson².* 1) Depts Medicine, Pathology and Oncology, SMBD-Jewish General Hospital and Montreal General Hospital, McGill University, Montreal, QC, Canada; 2) Depts Human Genetics, Epidemiology and Biostatistics, and Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA.

The outcome following the diagnosis of invasive breast cancer in carriers of *BRCA1* or *BRCA2* mutations is an important question. We addressed this by combining data from two studies that used a similar historical cohort design, where mutation status is established independently of vital status. We tested DNA extracted from pathology blocks from 507 Ashkenazi Jewish women who were diagnosed with invasive breast cancer at Memorial Sloan-Kettering Cancer Center, New York (n=305) or Sir M.B. Davis-Jewish General Hospital, Montreal (n=202) between 1980-1995 and followed up for a median of 111 months. The patient characteristics at the 2 centers did not significantly differ, except that the Montreal series did not include women diagnosed with breast cancer older than age 64. 60 women (11.8%) carried a *BRCA* mutation (46 *BRCA1*, 15 *BRCA2*, 1 patient with both). There were 80 deaths from breast cancer, 19 of these were among *BRCA* carriers (24%). At the median follow-up, the breast cancer-specific mortality rate was 35% in *BRCA* carriers and 14% in non-carriers ($P < .001$). Omitting the *BRCA2*+ cases did not significantly alter these findings ($P < .001$). In a multivariate Cox model, including tumor size, nodal status and *BRCA* status, the RR for death from breast cancer for *BRCA* mutation carriers was 2.6 (95% CI: 1.5-4.6, $P < .001$). Tumor size and nodal involvement also predicted outcome (RRs 2.7, $P < .001$ and 1.8, $P = .02$, respectively). The results did not significantly change when we excluded women (n=87) in the New York series who were diagnosed at 65 years of age or older. These results show that the presence of a *BRCA1/2* mutation is an independent marker for a poor prognosis following breast cancer. This will be important information for women who carry a *BRCA* mutation, are currently unaffected and are considering the preventive options.

Genetic classification of Fanconi anemia patients in the IFAR: low prevalence of complementation group F. S.D. Batish¹, H. Hanenberg², P.C. Verlander¹, R.L. Zampolin¹, O. Levan¹, A.D. Auerbach¹. 1) Lab of Human Genetics & Hematology, Rockefeller University, New York, NY; 2) Children's Hospital, Heinrich-Heine University, Dusseldorf, Germany.

Fanconi anemia (FA) is an autosomal recessive disorder characterized by cellular hypersensitivity to DNA crosslinking agents. Cell hybridization studies have revealed extensive genetic heterogeneity in FA, with eight complementation groups reported. The genes for complementation groups FA-C (*FANCC*), FA-A (*FANCA*) FA-G (*FANCG*) and FA-F (*FANCF*) have been cloned in that order. Elucidation of the DNA sequence and molecular organization of these genes have enabled us and others to detect mutations in the DNA of FA patients and their family members. Here we used functional complementation of EBV-transformed lymphoblastoid cell lines (LCLs) by retroviral vectors containing *FANCA* or *FANCF* to identify complementation groups. IFAR patients with known mutations in *FANCA* (123 probands), *FANCC* (53 probands) and *FANCG* (40 probands) were excluded from this study. Cell lines from 109 FA patients were tested for sensitivity to the clastogenic effect of DEB. Of these, 49 cell lines were DEB-resistant while 60 cell lines were sensitive. We had previously observed that while most FA patients are uniformly sensitive to the clastogenic effect of DEB in PHA-stimulated peripheral blood lymphocytes (PBLs), some FA patients are somatic mosaics, exhibiting both DEB-sensitive and resistant PBLs. The median percentage of DEB-resistant cells on cytogenetic testing of PBLs was 28 and 4 in patients with DEB-resistant and DEB-sensitive LCLs respectively. Of a total of 50 sensitive LCLs studied with retroviral vectors containing *FANCA* or *FANCF*, 40 were in FA-A, 3 were in group F and 7 were nonFA-A/nonFA-F. The later are under further investigation. Our data on the frequency of the different complementation groups indicates that FA-A (61%), FA-C(20%) FA-G (15%) and FA-F (1.5%) together account for approximately 97% of all FA cases. Thus the remaining genes not yet identified are quite rare. Mutation screening should now be feasible for most FA families.

The *HRAS1* minisatellite locus increases the risk of ovarian cancer in *BRCA1* carriers, but not in *BRCA2* carriers or sporadic ovarian cancer. *W. Chu*¹, *J. McLaughlin*², *C. Phelan*¹, *D. Cole*³, *H. Risch*⁴, *S. Narod*¹. 1) Centre for Research in Women's Health, Sunnybrook and Women's College Health Sciences Centre, Toronto, ON; 2) Div. of Epidemiology and Statistics, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON; 3) Dept. of Laboratory Medicine and Pathobiology, Banting Institute, Toronto, ON; 4) Dept. of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT.

Ovarian cancer is the second most common gynecologic malignancy in women and the fifth leading cause of cancer death in North America. Approximately 10% of ovarian cancers are attributable to mutations in *BRCA1* and *BRCA2*. The rare alleles of the minisatellite *HRAS1*-variable number of tandem repeats (VNTR) locus, located 1 kilobase (kb) downstream of the *HRAS1* proto-oncogene on chromosome 11p15.5, have previously been shown to increase the risk of ovarian cancer risk 2- to 3-fold in both *BRCA1* carriers and in sporadic ovarian cancer. We conducted a population-based study of incident cases of ovarian cancer in Ontario, diagnosed between 1995-1996, to determine the impact of the presence of rare *HRAS1* alleles on ovarian cancer risk. Using a PCR-based assay we typed the DNA for the *HRAS1* alleles from 355 patients, and from 132 ethnically-matched, population-based controls. The frequency of the four common alleles (a1, a2 a3 and a4) and rare alleles in the cases was respectively 0.56, 0.10, 0.12, 0.05 and 0.17, versus 0.56, 0.12, 0.11, 0.06, and 0.15 in the controls. At least one rare *HRAS1* allele was observed in 48% of the 21 *BRCA1* mutation-positive cases (O.R.=2.42, p=0.05) and in 17% of the 12 *BRCA2* mutation-positive cases (O.R.=0.53, p=0.34). Rare *HRAS1* alleles were observed in 28% of the 322 mutation-negative cases and in 27% of the 132 controls (O.R.=1.05, p=0.46). We did not observe an association with the rare *HRAS1* alleles and the age of cancer diagnosis. The results of this study confirms our previous observations that the *HRAS1*-VNTR locus may only increase ovarian cancer risk in ovarian cancer associated with *BRCA1* mutations, but refutes findings of an association in non-hereditary cancers.

HNPCC: Efficient diagnosis and management require a pluridisciplinary network. *C.D. Delozier-Blanchet¹, J-L. Blouin¹, M.A. Brundler², J-F. Egger¹, P. Hutter³, C. Soravia⁴.* 1) Div Medical Genetics, Geneva Univ Hospital; 2) Div Clinical Pathology, Geneva Univ Hospital; 3) Dept. Pathology, ICHV, Sion; 4) Div Digestive Surgery, Geneva Univ Hospital, Switzerland.

INTRODUCTION: Some 5-10 percent of colorectal cancers are associated with dominantly-inherited mutations, mostly in genes controlling mis-match repair (MMR). The diagnosis of Hereditary Non-Polyposis Colon Cancer (HNPCC, Lynch syndrome) has prognostic and treatment implications for affected individuals as well as for early detection in at-risk relatives. **GOAL:** To implement an efficient diagnostic, counseling and management network for HNPCC. **METHODS:** Over the past 2 years a hospital-based team made up of gastroenterologists, oncologists, surgeons, pathologists, clinical and molecular geneticists has been formed to deal with management of HNPCC. Families meeting the Amsterdam/Bethesda criteria have been offered molecular analysis of hMLH1 and hMSH2 throughout the period. During the past year two additional laboratory diagnostic tests have been developed: a 5-marker search for micro-satellite instability (MSI), and the immunohistochemical determination of presence/absence/quantitative variation in the MMR proteins. We now also offer these screening tests to individuals without convincing family histories but whose young age (<50) suggests a germline mutation. **RESULTS:** Overall, 3/15 tumors have shown MSI and 7/15 have demonstrated absence or quantitative reduction of one of the MMR proteins. Germline mutations have thusfar been identified in two patients; eight first-degree relatives are at risk. Analysis of tumors from both families showed high-level MSI and absence/reduction of hMLH1 or hMSH2 proteins in tumor tissue. Surgical or chemotherapeutic management decisions may be modified in some patients in light of such laboratory results. **CONCLUSIONS:** Development of a multi-disciplinary laboratory and clinical team facilitates optimal clinical management of families with suspected Lynch syndrome. The use of MSI and IHC allows more rapid para-clinical diagnosis and renders molecular investigation more time and budge-efficient, by targeting specific families for germline mutation research.

Estrogen receptor alpha and beta in colon cancer cells and growth effects of Raloxifene. Z.Q. Gao¹, Z.P. Gao¹, T. Zhang¹, A. Kairo¹, S. Burkholder¹, J. Fields², S. Ehrlich¹, B.M. Boman¹. 1) Medical Genetics, Thomas Jefferson University, Philadelphia, PA; 2) CA*TX inc, Gladwyne, Philadelphia, PA 19035.

Colorectal cancer is the third leading cause of cancer death in women. Epidemiological and experimental data suggest an involvement of estrogen in the development and progression of colorectal cancer. Studies on the expression of estrogen receptor (ER) in human colonic mucosa and cancer and the potential protective effect of postmenopausal hormone replacement on the incidence of colon cancer have been reported. Accordingly, we studied the expression of ER-alpha and beta mRNA in human colon cancer cells (RKO, LoVo, HT29 and HCT116) and evaluated the growth effects of the anti-estrogen agent raloxifene on these cell lines. Cells were cultured in phenol red-free medium containing charcoal-treated serum. The results from expression assay showed that four human colon cancer cell lines, RKO, LoVo, HT29 and HCT116 expressed ER-beta mRNA but not ER-alpha mRNA using reverse transcription PCR. Cell growth experiments demonstrated that raloxifene inhibited the growth of these four colon cancer cell lines, in a dose-dependent manner, as well as the growth of ER-positive human breast cancer cells MCF-7. However, these colon cancer cells were not influenced by estradiol E2. Genistein, a phytoestrogen that is similar to estradiol, has greater affinity for ER-beta than ER-alpha. Genistein at 10uM exhibited weak inhibitory effects on the growth of all these CRC cell lines, which is the same concentration that stimulated the growth of ER-alpha-positive MCF-7 breast cancer cells. These results coupled with previous studies indicate that ER-alpha and ER-beta may play different biological functions in colon cancer cells.

Risk assessment model for HNPCC and for germline MLH1 & MSH2 mutations based on age-at-colorectal cancer (CRC) diagnosis. *B.M. Boman*¹, *P. Watson*², *G. Fant*³, *J.Z. Fields*⁴, *G. Matika*¹. 1) Division of Medical Oncology and Genetics, Thomas Jefferson University, Philadelphia, PA; 2) Creighton University, Omaha, NE; 3) Walter Reed Army Medical Center, Washington, DC; 4) CATX INC., Gladwyne, PA.

Currently, no risk assessment models exist to estimate the likelihood of hereditary nonpolyposis colorectal cancer (HNPCC) or of germline mismatch repair gene mutations in the individual CRC patient; the revised Amsterdam Criteria (ACII) require a positive family history of cancer. Accordingly, we developed a model using "age at CRC diagnosis" as the sole criterion to determine the relative risk of having an MSH2 or MLH1 germline mutation or of HNPCC. This model can be applied to CRC patients who do not fulfill ACII or who have an unknown family history. Calculations were based on the differential age distributions of HNPCC versus all CRC. "Early age at CRC diagnosis" had high predictive value in the CRC patient population: the relative risk (RR) for HNPCC [$RR = (\text{Risk if age is} < \text{cutoff} / \text{Risk if age is} > \text{cutoff})$] peaked at age <55 with an RR = 105 (CI = 94-116). This high RR will detect many, if not most, HNPCC cases missed (by ACII) because of negative family history. To assist genetic counselors and clinicians in identifying HNPCC and to determine who should undergo genetic testing, a table was created to estimate the odds ratio (probability of disease / probability of no disease), for CRC patients at various ages, of a) HNPCC and b) MSH2 & MLH1 mutations. The table is based on modeling using estimates that MSH2 & MLH1 mutations are found in 2/3 of diagnosed HNPCC families & that overall sensitivity of ACII is ~50%. If a CRC patient is determined to be at high-risk (<55), we recommend molecular testing for microsatellite instability to eliminate false positives before DNA sequencing. Use of our "risk assessment model" will improve the clinician's ability to efficiently and cost-effectively diagnose HNPCC-affected CRC patients and their at-risk family members. This should lead to a significant reduction in cancer mortality in those carrying a germline, disease-causing mutation.

Genetic heterogeneity in hereditary paraganglioma (PGL): SDHD is the primary locus in imprinted PGL pedigrees. *B.E. Baysal^{1,2}, J.E. Willett-Brozick¹, E.C. Lawrence², C.M Drovdic², D. Myssiorek³, E.N. Myers⁴, R.E. Ferrell², W.S. Rubinstein^{5,2}.* 1) Dept Psychiatry Univ Pittsburgh Sch Med Pittsburgh PA; 2) Dept Hum Genet GSPH Univ Pittsburgh; 3) Dept Otolaryngol/Commun disorders, Long Island Jewish Med Ctr New Hyde Park NY; 4) Dept Otolaryngol Univ Pittsburgh Sch Med; 5) Pitt Cancer Inst.

PGL is characterized by the development of highly vascular, benign tumors in the head and neck. The oxygen-sensitive carotid body is the most common tumor site. PGL1 at chromosomal band 11q23 is the most common locus, although two more loci, PGL2 at 11q13 and PGL3 of unknown chromosomal location, have been implicated. PGL1 and PGL2 show exclusive paternal transmission suggesting that they are subject to genomic imprinting, whereas PGL3 shows biparental transmission. PGL1 has been recently uncovered as the SDHD gene, which encodes the small subunit of cytochrome b (cybS) in mitochondrial complex II (MTCII; succinate-ubiquinone oxidoreductase). MTCII is composed four subunits: a flavoprotein (Fp), an iron-sulfur protein (Ip), the large subunit of cytochrome b (cybL) and cybS. We assessed SDHD in nineteen "unrelated" PGL families, eighteen of which were ascertained from North America and one from Turkey. Fourteen of sixteen families that did not violate maternal imprinting (inactivation) had loss-of-function mutations in the SDHD gene. Haplotype analysis revealed eight of the thirteen American PGL families (61%) with SDHD mutations had the same P81L mutation and identical disease haplotypes (i.e., sharing a common ancestor). Three small families that violated imprinting, by showing maternal disease transmission, excluded SDHD by linkage and mutation analysis. Linkage analysis further excluded SDHA and SDHB, which encode Fp and Ip at 5pter and 1p33-35, respectively. Interestingly, all three families showed suggestive linkage to the SDHC locus, which encodes cybL at chromosomal band 1q21. However, no mutations could be detected in SDHC. These findings suggest that SDHD is the primary locus in PGL pedigrees that show maternal imprinting and that neither SDHD nor other MTCII loci are involved in families that violate maternal imprinting.

Genetic Studies of Peutz-Jeghers Syndrome. *C.I. Amos¹, M.L. Frazier¹, P.M. Lynch¹, T. McGarrity²*. 1) Department of Epidemiology, U.T.Anderson Cancer Ctr HMB, Houston, TX; 2) Division of Gastroenterology, Penn State College of Medicine, Hershey, PA.

We have assembled and are studying a large cohort of families and individuals with Peutz-Jeghers syndrome. Our initial studies among the relatives of index cases indicate that the median time to onset for gastrointestinal symptoms in our cohort is 10 years of age and the time to onset for first polypectomy is 11 years of age. End to end sequencing of STK11, which has been implicated as a cause for Peutz-Jeghers syndrome, showed mutations in 69% (9/13) of individuals from families with PJS. We were unable to identify mutations in the majority of sporadic cases of Peutz-Jeghers syndrome, with only 30% (3/10) showing detectable mutations in STK11. Mutations in the first exon appeared to confer a less severe phenotype than mutations in other exons.

A High Prevalence of *BRCA2* Mutations in Unselected Breast Cancer Patients from the Philippines. A. Liede¹, M.L. de Leon Matsuda², A. Tan³, E. Kwan¹, A. Borg⁴, S.A. Narod¹. 1) Centre for Research in Women's Health, University of Toronto, Toronto, ON, Canada; 2) Department of Surgery, Philippine General Hospital, Manila, Philippines; 3) Department of Surgery, Davao Medical Center, Davao City, Philippines; 4) Department of Oncology, Lund University Hospital, Lund, Sweden.

Breast cancer incidence varies ten-fold throughout the world, with the highest rates in North America and Europe. The highest breast cancer rates in Asia are reported for the Manila Cancer Registry in the Philippines, with an age-standardized rate of 47.7 per 100,000 per year. The contribution of hereditary factors to these elevated rates has not been investigated. Here we report the results of *BRCA1* and *BRCA2* mutation analysis of 375 unselected breast cancer patients from the Philippines. Subjects include women undergoing examination of a breast lump at the Philippines General Hospital in Manila and at the Davao Medical Center in Davao City. The mean age of breast cancer diagnosis was 43.8 years. Molecular analysis identified 11 *BRCA2* mutations and one *BRCA1* mutation among the series of patients. The *BRCA2* 4265delCT and 4859delA were found in six unrelated breast cancer patients. Genotype analysis was performed using 7 polymorphic markers on 13q to explore the possibility of founder effects in the Philippines; the 4265delCT mutation appears to represent an independent origin from a Swedish family previously described. The age of onset of cases with identified *BRCA* mutations did not differ from that of the general case series. Five of the 12 patients with *BRCA* mutations had no family history of breast or ovarian cancer. Age and family history do not appear to be strong predictors of genetic predisposition to breast cancer in the Philippines. Our data suggest that *BRCA2* is the more significant gene for breast cancer patients in the Philippines, due in large part to the presence of two founder mutations. Because a high proportion of breast cancers in the Philippines are diagnosed in women younger than 50 years, hereditary factors may contribute to breast cancer in the Philippines to a greater degree than in North America or Europe.

A Genetic Epidemiological Study of Carcinoma of the Fallopian Tube. *R. Nedelcu¹, S. Aziz², G. Kuperstein⁴, B. Rosen³, J. McLaughlin^{1, 5}, S.A. Narod^{1, 4}.* 1) Familial Ovarian Cancer Clinic, Princess Margaret Hospital, Toronto, Canada; 2) Public Health Sciences, University of Toronto, Toronto, Canada; 3) Department of Obstetrics and Gynecology, The Toronto Hospital, Toronto, Canada; 4) The Centre for Research in Women's Health, Sunnybrook and Women's College Health Sciences Centre, Toronto, Canada; 5) Samuel Lunenfeld Research Institute, Toronto, Canada.

To evaluate the importance of genetic factors in the etiology of fallopian tube cancer, all pathologically-confirmed cases of fallopian tube cancer diagnosed in Ontario from 1990 to 1998 were identified from the records of the Ontario Cancer Registry. Living patients were approached to provide information about their family history and to provide a blood sample for testing for mutations in BRCA1 and BRCA2. A modest increase in the risk of ovarian cancer (relative risk (RR) = 2.2; 95% confidence interval (CI) = 0.4, 6.3) and of early-onset breast cancer (RR = 2.4; 95% CI = 0.6, 6.1) was observed in the first-degree relatives of the fallopian cancer cases. Four of the 44 cases were positive for a mutation in BRCA1 (9%) and two were positive for a BRCA2 mutation (5%). One of the six mutation carriers had a strong family history of breast and ovarian cancer, and three carriers had a modest family history. Three of the 44 cases were Jewish, and of these, two carried a founder mutation characteristic of this population. Fallopian tube carcinoma should be considered to be a clinical component of the hereditary breast-ovarian cancer syndrome, and may be associated with BRCA1 and BRCA2 mutations. Genetic evaluation should be offered to women who present with fallopian tube carcinoma. It is important to consider the risk of fallopian tube carcinoma when prophylactic oophorectomy is performed in high-risk women.

Multiple primary cancers in south-east England: relevance to colon cancer genetic susceptibility. *S.V. Hodgson¹, H.S. Evans², H. Møller², D. Robinson², C.M.J. Bell², C.M. Lewis¹*. 1) Div Medical & Molec Genetics, GKT School Medicine, London, England; 2) Thames Cancer Registry, Div Oncology, GKT School Medicine, London, England.

A number of genes have been identified in which inherited mutations can cause a predisposition to cancers at multiple sites. For example, hMSH2 and hMLH1 confer a high risk of colorectal, uterine and other cancers. This study characterises the occurrence of subsequent primary cancers in patients diagnosed with colon cancer, to explore the possibility of a common aetiology between different cancer sites and identify novel genetic associations.

Registrations of patients with a first cancer diagnosed between 1961 and 1995 were extracted from the database at Thames Cancer Registry, SE England. Expected numbers of subsequent cancers were compared with observed numbers in the study population and relative risks (RR) calculated. Analysis was stratified by sex and age at diagnosis.

Analysis of a cohort of over 80000 patients with primary colon cancer identified 4.5% with at least one subsequent tumour. Preliminary results show that women with a young onset of colon cancer (<70y) have a significantly increased risk of developing cancer of the small intestine(RR 2.9),rectum (RR 1.3),breast(RR 1.2),uterus(RR 1.8),ovary(RR 2.6) and eye(RR 3.5). Two cancer sites were significantly increased in women with a first diagnosis of colon cancer after 70: small intestine(RR 2.6) and ovary(RR 1.8). Men diagnosed with colon cancer before 65 had a significantly increased risk of developing cancer of the small intestine(RR 3.5), colon(RR 2.0) and rectum(RR 1.6). Men diagnosed with colon cancer at an older age did not show a significantly increased risk of cancer at any site.

We have shown an association between extra-colonic cancers at increased risk in HNPCC (rectum,ovary,uterus,small intestine) and some novel associations (breast,eye). These may represent genetic associations as they occur primarily after diagnosis of colon cancer at a young age.

Multiple measures of genomic instability in a familial breast cancer patient versus a sporadic breast cancer

population. *J.J. Latimer*^{1,2}, *W.S. Rubinstein*^{1,2}, *V.G. Vogel*^{1,2}, *S.G. Grant*^{1,2,3}. 1) OB/GYN & Women's Health, Magee-Womens Research Institute, Pittsburgh, PA; 2) University of Pittsburgh Cancer Institute, Pittsburgh, PA; 3) Center for Environmental and Occupational Health, Pittsburgh, PA.

We have previously observed that newly diagnosed breast cancer patients have higher levels of somatic mutation than age matched controls and that there is a characteristic loss of Nucleotide Excision DNA Repair (NER) in the breast tumors themselves. We have begun to analyze our patient population for evidence of familial breast cancer syndromes, in order to determine whether this subset of patients has a distinctive pattern of genomic instability. The first such patient was age 54 at the time of her diagnosis and surgery. She had been diagnosed with stage II ductal carcinoma with positive nodes. The Berry-Parmigiani-Aguilar model was used to estimate the probability that she carried a germline *BRCA1* or *BRCA2* mutation. Based on her diagnosis and a mother with bilateral breast cancer at age 33 and death at age 38, the probability of germline mutation was calculated to be 76%. This patient had a bone marrow somatic mutation frequency of 41.4×10^{-6} , approximately twice that of a large population of disease free individuals ($19.9 \pm 0.9 \times 10^{-6}$), but indistinguishable from those of our incident breast cancer population ($47.7 \pm 14.6 \times 10^{-6}$, N = 47). Thus, it is unlikely that our observed increase in somatic mutation in breast cancer patients is driven by the approximately 10% that are carriers of mutations in the *BRCA1* or *BRCA2* genes, despite the fact that these genes have been implicated in DNA repair processes. Direct analysis of NER capacity in primary explants of this patient's tumor tissue using the UDS assay revealed a significant loss of DNA repair retaining only 15% of normal levels, whereas the overall population of similarly staged tumors showed an average level of DNA repair of 50% normal. This is the lowest level of NER we have observed for stage II breast cancer.

VHL gene testing: qualitative versus quantitative molecular analysis. *M. Martella¹, R. Polli¹, G. Opocher², A. Murgia¹.* 1) PEDIATRICS, UNIVERSITY OF PADUA, PADUA, PD, ITALY; 2) MEDICAL AND SURGICAL SCIENCES, UNIVERSITY OF PADUA, PADUA, PD, ITALY.

We perform mutation scanning of the von Hippel-Lindau (VHL) disease gene with the combined use of quantitative Southern blot, and PCR-SSCP analysis of the entire coding sequence, followed by direct sequencing of altered fragments. This strategy has led to the identification of disease-causing mutations in 21 out of 22 patients with clinical von Hippel-Lindau disease. VHL germ-line mutations were detected in patients with isolated tumors and negative family history: 2/21 cases of pheochromocytoma, 2/9 patients with CNS haemangioblastoma and 1/11 cases of isolated retinal haemangioma. One intronic alteration and one apparent polymorphic variant are currently being characterized. 4 new disease-causing mutations have been also detected. We have identified one case of somatic mosaicism (Murgia et al. 2000) in which the mutation was present with a ratio of approximately 1/12-1/15 mutated versus normal cell lines. Very often direct sequencing is viewed as a more efficient and complete method if compared to other mutation-scanning techniques. In this case the mosaic alteration, visible as a very faint shifted band at the SSCP analysis was completely undetectable and would have been missed by just sequencing the amplicon. von Hippel-Lindau is a well-known monogenic disease, the protocol we use has allowed detecting mutations in 95% of the VHL patients tested. The identification of a VHL mosaic individual and the fact that the only apparently molecular negative clinically affected patient of our series is a sporadic case, suggested the need to modify the test we have been using for our molecular screening in order to increase its quantitative sensitivity and ensure the maximum possible chance to recognize the presence of low-grade mosaicisms. A complete and reliable test is essential for the correct interpretation of the clinical significance of a negative molecular result in individuals affected by VHL related tumors who do not meet strict criteria for clinical diagnosis of the disease. This work is supported by: Ricerca Sanitaria Finalizzata Regione Veneto No. 930/02/99.

Mutations in the RUNX1/CBFA2/AML1 gene in dominant familial platelet disorder with predisposition to acute myelogenous leukaemia (FPD-AML). *J. Michaud¹, K.F. Benson², W.H. Raskind², C. Rossier¹, S.E. Antonarakis¹, M. Horwitz², H.S. Scott^{1,3}.* 1) Division of Medical Genetics, Geneva University Medical School, Switzerland; 2) Division of Medical Genetics, University of Washington School of Medicine, USA; 3) Genetics and Bioinformatics Group, Walter and Eliza Hall Institute, PO, Royal Melbourne Hospital, 3050, Victoria, Australia.

FPD-AML is an autosomal dominant familial platelet disorder characterized by thrombocytopenia and a propensity to develop acute myelogenous leukaemia. The identification of genes responsible for rare familial leukemias may afford insight into the mechanism underlying more common sporadic occurrences, and particularly the mechanisms initiating the multistep leukemogenic pathway. Consistent with other studies, we studied 3 families with FPD-AML showing linkage to chromosome 21q22.1. The gene for the RUNX1/AML1/CBFA2 transcription factor, previously identified as the target of translocations and somatic point mutations in sporadic AML, maps to 21q22.1. Here we describe two novel heterozygous point mutations in RUNX1/AML1/CBFA2 in 2 of 3 FPD-AML families. Both mutations are in the part of the gene encoding the DNA binding runt domain. They are; i) a missense mutation, K83E, in exon 3, a residue known to destroy DNA binding if mutated, and ii) a one-base deletion in the splice donor site of exon 4, IVS4+3delA, which results in abnormal splicing, frameshifts and prematurely truncated proteins. As all coding regions of AML1 in affected individuals from the third family are normal, a gross deletion is likely. Our studies, in agreement with 6 recently published FPD-AML mutations, indicate that haploinsufficiency of RUNX1/AML1/CBFA2 causes FPD-AML.

Ataxia telangiectasia: a model for understanding DNA damage response. *K. Jen, J. Watts, V.G. Cheung.*

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Ataxia telangiectasia (AT) is an autosomal recessive disorder resulting from mutations in the gene ataxia telangiectasia mutated (*ATM*) located on chromosome 11q22-23. AT patients develop a wide range of clinical features including cerebellar ataxia, oculocutaneous telangiectasia, predisposition to malignancies, and immune disorders. In addition, AT cells display acute sensitivity to ionizing radiation (IR) and are unable to properly activate cell cycle checkpoints in response to DNA damage resulting in overall chromosome instability.

Differential gene expression between lymphoblasts from AT patients and normal lymphoblasts was studied as a screen for genes and pathways involved in IR stress response. Approximately 2000 genes were examined through microarray analysis of which approximately 100 were chosen because of their role in DNA damage response. The basal gene expression levels of AT and normal cells along with gene expression levels of the two cell types after IR exposure at 3Gy (300rads) were analyzed and compared. Gene expression profiles were taken for 2hr, 6hr, 12hr, and 24hr post irradiation. Groups of genes identified to be significantly different in expression pattern between AT and normal cells were then confirmed by quantitative RT-PCR. Normal cellular adaptation to IR was also examined by comparing gene expression profiles of normal cells at various time points. In this presentation, we will show preliminary data from this study.

XRCC1-R399Q, XRCC3-T241M, XPD-K751Q genotypes and DNA-adduct levels in traffic exposed workers from Florence. *G. Matullo¹, M. Peluso³, A. Russo², S. Guarrera¹, S. Carturan¹, A. Munnia³, G. Masala², C. Saieva², S. Polidoro¹, A. Piazza¹, P. Vineis⁴, D. Palli².* 1) Dip. di Genetica Biologia e Biochimica, Univ. Torino; 2) Epidemiology Unit, CSPO, A.O. Careggi, Florence; 3) Unit of Experimental Oncology, IST, Genoa; 4) Dipartimento di Scienze Biomediche e Oncologia Umana, Univ. Torino, Italy.

We measured DNA adducts in peripheral leukocytes in two groups of residents in Florence, who participated into the prospective study EPIC-Italy, a section of a larger European project. Overall, 13,597 volunteers, aged 35-64 years, were enrolled in the period 1993-1998: a random sample of 100 subjects was drawn from the cohort and compared with a group of 114 workers selected because exposed to vehicle traffic pollution. DNA adducts were determined in peripheral leukocytes by using 32P-postlabelling technique and the following three polymorphic DNA repair genotypes were investigated by PCR methods: XPD-K751Q, XRCC1-R399Q, XRCC3-T241M. DNA adducts were detected in approximately 85.0 % of both groups. Overall, crude mean levels tended to be higher among traffic workers (14.0 ± 1.3 vs. 11.1 ± 1.3 adducts per 10^9 normal nucleotides, $p = 0.10$); differences were statistically significant among never- and light current-smokers ($p < 0.03$). In both groups, urban residents tended to show higher levels than those living in the surrounding area, and a seasonal trend emerged with adduct levels being highest in summer and lowest in winter months. Two DNA repair genotypes (XRCC1-3) were not significantly related to levels of adducts. Exposed workers with at least one XPD-Gln751 allele had significantly higher levels in comparison to workers with two common alleles. A multivariate analysis showed a significant positive association between occupational exposure and higher levels of adducts (OR 2.1; 95%CI 1.1-4.2) after adjustment for several confounders. Overall, these results suggest that traffic workers and the Florence general population are both exposed to high levels of genotoxic agents related to vehicle emissions. Photochemical pollution in warmer months might be responsible for particularly elevated levels of genotoxic damage in this metropolitan area.

The impact of breast cancer risk assessment and education for women at average and moderately increased risk.

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A research program was established in 1998 to evaluate the impact of risk assessment for breast cancer in a suburban medical center. This program was free and anonymous and enrolled any woman interested in learning about her breast cancer risk. A woman's risk was assessed by a medical oncologist/genetic counselor/nurse educator team using reported family history, and the Gail and Claus statistical models. Women were stratified into three risk categories: average (A), somewhat higher than average (SHA), and high (H) risk. Over an 18-month period, 46 women (mean age, 48.6) were evaluated and assigned to either the A or SHA risk categories. Each woman met with a nurse educator working in an outpatient education suite for a 1 to 2 hour session to discuss her risk assessment, the appropriate guidelines for breast surveillance, and any specific concerns. Self-breast exam (SBE) was also demonstrated using models allowing the woman to practice. Pre- and post-counseling questionnaires were administered to assess breast cancer risk perception, anxiety about developing breast cancer, breast screening practices, and general knowledge about breast cancer risk. About 55% of women incorrectly assessed their risk category on the pre-test questionnaire. Consistent with previous studies, 75% of these overestimated their risk. Following counseling, 75% of all women who assessed their risk incorrectly on the pre-test questionnaire, correctly adjusted their risk as measured on the post-test questionnaire. Nearly all women reported having benefited from their participation in the program. We conclude that breast cancer risk assessment and education are useful for women at average and moderately increased risk for breast cancer, as well as for high risk women. Aside from reinforcing appropriate breast surveillance, they are effective in helping women to form a more accurate picture of their risk with the goal of reducing anxiety caused by an overestimation or lack of knowledge about their true risk.

Age-at-colorectal-cancer-diagnosis (ACCD) correlates with microsatellite instability (MSI). *G.L. Matika, S. Burkholder, D.G. Rose, R. Fishel, T. Edmonston, R.D. Fry, S.M. Ehrlich, B.M. Boman.* Kimmel Cancer Center, Thomas Jefferson University Hospital, Philadelphia, PA.

Identifying carriers of hereditary nonpolyposis colorectal cancer (HNPCC) traits, who account for up to 10% of all colorectal cancer (CRC) cases, can save lives & health care dollars. But, current gene testing of patients for germline HNPCC-causing mutations, DNA sequencing, is costly & time consuming. And MSI, which is found in tumors in the vast majority of HNPCC patients with germline MSH2 & MLH1 mutations is highly sensitive, but not specific, being found in 10-20% of sporadic CRCs. Clinical diagnostic tests e.g. the Amsterdam Criteria (AC), the Bethesda Criteria (BC), & other family history (FH) based criteria also have short comings including limited sensitivity & specificity. Only two-thirds of AC(+) patients will have a detectable germline mutation (mostly MSH2 or MLH1). Because using FH will miss many HNPCC cases, one of us (BB) developed a risk assessment model based on ACCD as the sole clinical criterion for predicting the HNPCC trait and MSH2/MLH1 germline mutations. Since MSI is a useful pretest having high sensitivity for MSH2/MLH1 mutations, in the present study we looked for a correlation between ACCD and MSI status. MSI was analyzed on 165 CRCs using the NCI-recommended panel (Cancer Res 58:5248-57) to subclassify tumors as microsatellite stable, MSI-low, or MSI-high (MSI-H). Results showed a positive correlation between MSI-H status and ACCD ($Rho=0.67$; $p=0.05$). MSI status of CRCs correlated variably with other criteria: AC(+) (76% MSI-H), BC(+) (28%), familial CRC (16%), & negative/unknown FH status (0%). Because young ACCD predicts MSI-H, this age criterion appears to have value for detecting HNPCC, especially if FH-based criteria fail to diagnose HNPCC. In our previous study, "ACCD < 55" was the optimal cutoff for HNPCC diagnosis in AC(-) patients. Our current series indicates that 67.5% of CRC patients <55 will not have MSI-H tumors. This shows the value of MSI as a follow up test to ACCD because among younger CRC (<55) patients there will be a substantial number of patients (67.5%) who are false positives & will not need DNA sequencing for MSH2/MLH1.

Stem cells and colorectal cancer development: Study of crypt colonocyte changes in familial adenomatous polyposis (FAP). *G. Kandimir¹, J. Palazzo², A. Kovatich², J.Z. Fields³, O.A. Runquist⁴, T. Zhang¹, B.M. Boman¹.* 1) Division of Medical Genetics, Thomas Jefferson University, Philadelphia, PA; 2) Department of Pathology, Thomas Jefferson University, Philadelphia, PA; 3) CATX INC., Gladwyne, PA; 4) Department of Chemistry, Hamline University, St. Paul, MN.

The mechanism underlying the initiation of colorectal cancer (CRC) at the cellular level remains unknown even though the first genetic event (germline APC mutation) and earliest tissue change (abnormal crypt proliferation) are characterized in FAP patients. Accordingly, we developed a mathematical model of cell proliferation, differentiation and apoptosis in the colonic crypt that is based on a set of differential equations. Systematic perturbations were used to determine which model parameters could simulate the proliferative shift. Across many perturbations, only increasing the stem cell (SC) number simulated the biological FAP data (compared to healthy controls); changes in rate constants did not. This theoretical evidence suggests that APC is a regulator of SC division and that APC mutation leads to an increased number of SC located in the crypt base. To study this mechanism using biologically designed experiments, we used immunohistochemistry to analyze the expression of bcl-2, survivin, & Ki-67 as markers for crypt base cells in normal colonic epithelium and adenomas from FAP patients (n=10). In FAP adenomas the proportion of cells expressing these 3 markers was increased compared to FAP normal colonic epithelium. Given that FAP adenomas are known to have loss of the second wild-type APC allele, our immunohistochemical results are consistent with our modeling data because both findings suggest that APC mutation leads to abnormal SC division favoring SC renewal and expansion of the crypt base cell population. Overall, these findings support the idea that APC mutation in the colonic SC is the critical event for tumorigenesis and that the SC is the cell of origin for clonal expansion leading to colonic adenomas.

Prevalence of founder *BRCA1* and *BRCA2* mutations in French Canadian women with breast cancer unselected for age or family history. *N. Hamel*¹, *P. Ghadirian*², *A.-J. Paradis*^{1,2}, *A. Robidoux*², *C. Potvin*², *J. Cantin*², *G. Kuperstein*³, *P.N. Tonin*¹, *J. Deschênes*¹, *P.O. Chappuis*¹, *W.D. Foulkes*^{1,2}. 1) Depts Medicine, Oncology and Pathology, Montreal General and Jewish General Hospitals, McGill University, Montreal, QC, Canada; 2) CHUM-Hôtel-Dieu, University of Montreal, QC, Canada; 3) Centre for Research in Women's Health, University of Toronto, Toronto, ON, Canada.

Founder mutations in the breast cancer susceptibility genes *BRCA1* and *BRCA2* have been found in selected French Canadian (FC) breast and ovarian cancer families (Am J Hum Genet 1998; 63: 1341-51). The prevalence of these mutations in unselected breast cancer diagnosed in FC women and the clinico-pathological features of these cases is currently unknown. We studied incident female first primary breast cancers diagnosed before the age of 80 years identified through the Dept of Pathology of Hôtel-Dieu, CHUM, Montreal, diagnosed between 07/96 - 04/98. The FC ethnicity was defined as having FC ancestry in at least 3 grandparents. 193 patients were eligible and 128 (66.3%) provided blood for the genetic testing. A pedigree was drawn with detailed information on the family history of cancer. Genomic DNA was screened for the 7 FC founder *BRCA1/2* mutations (*BRCA1*: C4446T, 3768insA, 2953del3+C; *BRCA2*: 8765delAG, G6085T, 2816insA, 6503delTT). Four (3.1%; 95% CI: 0.86-7.8%) women were identified as carrying a germ-line mutation, all in *BRCA2* (2x 8765delAG, 6503delTT, G6085T). The mean age at diagnosis was 51 years (range: 49-53). *BRCA2*-related breast cancers were invasive ductal (2/4) and lobular (2/4) carcinomas, of histological grade 1 or 2, axillary nodal involvement was present (3/4) despite a small tumor size (<20 mm), and ER was strongly expressed (3/3). Among these 4 cases, 2 reported a strong family history of breast cancer and 2 had unremarkable family history. The prevalence of the 7 *BRCA1/2* founder mutations screened among this series of breast cancer in unselected women of FC origin is estimated to be 3.1%, but much larger studies from different regions of Quebec will be required for more precise estimates of prevalence and penetrance of these founder mutations.

Isolation of four novel genes from human colonic epithelial cells using bHLH transcription factor as bait in a yeast two-hybrid system. *A. Kairo, L. Wang, Z.Q. Gao, Z.P. Gao, T. Zhang, S. Burkholder, S.M. Ehrlich, B.M. Boman.* Medical Genetics, Thomas Jefferson University, Philadelphia, PA.

Transcription factors (TFs) play a central role in regulating gene expression that is important in differentiation, development, and tumorigenic processes. For example, Id1 TF overexpression causes colonic adenoma development in murine models for colorectal cancers (CRC). The aim of this study was to isolate novel factors that interact with the basic-helix-loop-helix (bHLH) TF. The conserved domain of the bHLH binds to other TFs, modulating gene expression and cell proliferation. The importance of identifying specific TFs from colon is: 1) regulation of TFs is likely to be important in colonocyte differentiation, colon development, and disease (e.g. CRC) and 2) novel TFs might help identify biomarkers for CRC and new therapeutic targets. Proteins that interact with bHLH can be TFs themselves. We used a yeast two-hybrid screen to detect proteins that interact with bHLH. Hence, the bHLH motif (nucleotides 1528-1962), which encodes a polypeptide that attaches to the E12-box promoter region, was cloned into a pGBT9 vector. Our cDNA libraries from normal (fresh mucosa) and malignant (HCT116 line) human colonic epithelial cells (cloned in pGAD10) & the bHLH bait plasmid was co-transfected into yeast HF7c cells. Using the HCT116 library, 4 genes were isolated: CATX-11 (1800 bp); CATX-13 (620 bp); CATX-14 (1100 bp); CATX-15 (1900 bp). Full-length clones of CATX-11 & CATX-15 have been isolated. Initial homology data showed that CATX-11 shares high sequence homology with other cDNAs reported to be stage specifically expressed in the trophoblast stage during development. CATX-13 & CATX-15 showed no significant homology to any known gene, indicating that they are novel. CATX-14 showed weak homology to the mouse TLM oncogene. In pilot studies using RT-PCR, CATX-15 was not detected in the library from normal colon, whereas CATX-13 was detected. Together our data suggests that these four genes are 1) novel 2) specifically expressed in the colonic epithelial cells 3) possibly unique TFs and 4) likely to be important in colonic cell biology.

The Bloom syndrome protein (BLM) and TopIIIa colocalize and are found together with BRCA1 and hRAD51 at telomeres in ALT pathway immortalized human cells. *M.S. Meyn^{1, 2}, D.J. Stavropoulos^{1, 2}.* 1) Dept Genetics, Hosp Sick Children, Toronto, ON, Canada; 2) Dept Molecular and Medical Genetics, Univ of Toronto, Toronto, ON, Canada.

Bloom syndrome is characterized by growth retardation, immune defects, cancer, infertility, and increased genomic instability. It is caused by mutations in the *BLM* gene, a RecQ-like DNA helicase. The *BLM* homologs, *SGS1* and *RecQ*, interact physically and functionally with topoisomerase III. As part of a study of telomere maintenance, we performed immunohistochemical analyses of BLM, TopIIIa, BRCA1 and hRAD51 proteins in human cells. These proteins localized to telomeres in immortalized cells that lack telomerase and maintain telomere length via an alternative pathway (ALT).

BLM formed nuclear foci that co-localized with telomeres in ~70% of interphase cells from two ALT fibroblast lines, WI38-VA13 and GM00847. This colocalization was not seen in a telomerase-positive fibroblast line (MRC5) or in a primary fibroblast line (WI38). TopIIIa co-localized to telomeric foci in 29-50% of cells from the two ALT lines, but colocalization was not seen in MRC5 or WI38 cells. TopIIIa co-localization with telomeres occurred only in cells in which BLM co-localized as well. We also observed colocalization of BRCA1 and hRAD51 to telomeric foci only in ALT cells. We confirmed the recently published result that BLM colocalizes to promyelocytic leukemia (PML) bodies in both telomerase positive and ALT cells. We found that TopIIIa also localized to PML bodies in a normal fibroblast cell line, MRC5, but not a *BLM*^{-/-} cell line, GM08505B.

Our results provide direct *in vivo* evidence for an association between BLM and TopIIIa. The localization of TopIIIa to telomeres and PML bodies appears to be dependent on functional BLM. The finding that BLM, and TopIIIa colocalize with the recombination proteins BRCA1 and hRAD51 to telomeric foci only in ALT cells suggests that these proteins may be involved in the ALT pathway of telomere maintenance and provides evidence that, in human cells, the ALT pathway may involve homologous recombination.

Frequency of glutathione-S-transferase theta (GSTT1) deletion in Mexican patients with lung cancer. G.M.

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Glutathione-S-transferase (GST) is a dimeric isoenzyme who participated in the desactivation of carcinogen, toxic reactives such as the detoxification of several tobacco smoke derived carcinogens, and xenobiotics, and it catalized the reducing reaction of glutathione of many organic compounds to thioester. Is is thus conceivable that deficiency in GST activity due to homozygous deletion of GSTT1 gene (the null genotypes) may modulate susceptibility to smoking, inducing to lung cancer. The distribution of the GSTT1 deletion gene in healthy controls and lung cancer patients were evaluated using peripheral blood DNA from 52 samples of lung cancer patients and 178 samples of healthy controls (41 nonsmokers, 63 passive smokers and 74 smokers). Increasing lung cancer risk was related to the GSTT1 null genotype when were compared between groups, patients shown significative difference when it was compared with controls: non smokers ($p=0.007$), passive smokers ($p=0.019$) and smokers ($p=0.007$). Our lung cancer displays the gene deletion in 21%, among population controls, the proportions are: among nonsmokers not exposed to passive smoking 2.3%, among nonsmokers who are passive smokers 6.3% and among ever smokers 5.4%. These results shown a tendency to presence the deletion GSTT1 gene among lung cancer in Mexican population when compared when were compared with healthy controls.

The role of *ATM* heterozygosity in cancer susceptibility and genomic instability. *S.N. Teraoka*^{1,2}, *K.E. Malone*³, *E.A. Ostrander*³, *J.R. Daling*³, *D. Willerford*², *M. McKay*⁴, *J. Bernstein*⁵, *P. Concannon*^{1,2}. 1) Virginia Mason Research Ctr, Seattle, WA; 2) University of Washington School of Medicine, Seattle, WA; 3) Fred Hutchinson Cancer Research Center, Seattle, WA; 4) Peter MacCallum Cancer Institute, Melbourne, AUS; 5) Mount Sinai School of Medicine, New York, NY.

ATM, the gene mutated in the autosomal recessive, cancer-prone syndrome ataxia-telangiectasia, participates in the response to DNA damage caused by ionizing radiation. *ATM* is also required in processes involving joining of DNA double strand breaks, such as mitotic and meiotic recombination. A-T patients are highly susceptible to cancer, especially lymphoma and leukemia. *ATM* heterozygotes are also reported to be at increased risk for breast cancer. We screened for *ATM* mutations among breast cancer cases with early-onset (diagnosed by age 35) or family history, and observed an increased frequency of missense mutations in cases (11/142; 7.7%) versus age-matched controls (1/80; 1.2%). Three of these cases with *ATM* missense mutations also had *BRCA1* or *BRCA2* mutations. Since *ATM* has been shown to associate with and phosphorylate *BRCA1*, it is possible that mutations in these two genes in a common pathway might interact and contribute to disease susceptibility. Therefore, we are extending our studies by screening for *ATM* heterozygosity (via denaturing HPLC) in breast cancer cases with *BRCA1* or *BRCA2* mutations. We are also screening 91 lymphoma cases and an equivalent number of controls for *ATM* heterozygosity. Further, we are exploring the connection between *ATM* and radiation sensitivity in two studies. In the first, we are screening for *ATM* mutations in 70 patients who had serious complications arising from radiation therapy. The second study is a recently initiated collaborative project that will compare the *ATM* status of 700 women with contralateral breast cancer and 1400 women with unilateral breast cancer in order to examine the interaction between *ATM* status and radiation therapy on the risk of developing a contralateral breast tumor.

Gender and Smoking Modify the Risk for Colon Cancer in the Presence of Putative Rapid NAT2 Acetylating Activity. *A.M. Weiss, J.S. Witte, D.A. Sirko-Osadsa, M.C. MacMillen, S.D. Markowitz, G.L. Wiesner.* Case Western Reserve University and University Hospitals of Cleveland, Cleveland, OH.

The N-acetyltransferase 2 gene (NAT2) encodes a polymorphic enzyme in the acetylation pathway of ingested carcinogens. NAT2 genotypes encoding putative rapid acetylation activity have been associated with a 2 to 3-fold increased risk for colorectal cancer (CRC), the third most common adult malignancy. 49 sibships, with at least one affected and one unaffected sibling, from the CWRU Colon Neoplasia Sibling Study were genotyped for NAT2 by PCR-RFLP and analyzed using conditional logistic regression to determine whether putative acetylation activity was associated with colon neoplasia. Unaffected siblings were negative for adenomatous polyps or CRC on colonoscopy or flexible sigmoidoscopy. Overall, 75/164 (45.7%) siblings affected with CRC or adenomatous polyps carried a rapid NAT2 genotype. A 2-fold increased risk was found in siblings with a rapid genotype compared to siblings with a slow genotype (OR=2.24, p=0.07). Rapid NAT2 genotypes were found 6 times more often in siblings affected with CRC compared to siblings affected with adenomatous polyps (OR=5.9, p=0.10). While not statistically significant, these results are similar to those of other studies. Analysis of gender, however, showed significant differences in CRC risk. Females with a rapid genotype were more likely to be affected with colon neoplasia compared to females with a slow genotype (OR=6.25, p=0.02). This effect was not seen for male siblings (OR=1.26, p=0.78), and may reflect hormonal influences on the acetylation pathway. Our analysis also suggests a possible effect of smoking on CRC risk in the presence of a rapid NAT2 genotype. Surprisingly, non-smokers with a rapid genotype were 9 times more likely to be affected with colon neoplasia compared to smokers with a rapid genotype (OR=9.14, p=0.04). We hypothesize that gender and smoking status may modify the risk of colon neoplasia by altering the activity of the acetylation pathway. Ongoing studies of 145 affected and unaffected sibling pairs will confirm these results and help us gain an understanding of the role of NAT2 on the risk for colon neoplasia.

BRCA1/2 Testing in Newly Diagnosed Breast Cancer Patients: Preliminary Data on Interest and Surgery Decisions. *B.N. Peshkin, B. Brogan, C. Isaacs, C. Lerman, M. Schwartz.* Cancer Control, Georgetown University, Washington, DC.

Breast cancer (BC) patients who have a BRCA1/2 mutation have up to a 65% risk of contralateral BC, and an elevated risk of ipsilateral BC. No data exist about the most effective surgical treatment for such women, but BRCA1/2 results may allow newly diagnosed high-risk women to make more informed decisions about surgery and reconstruction. Pilot data from 75 BC patients indicated a high level of interest in genetic testing: 88% reported that they would have wanted genetic testing at the time of diagnosis and 31% would have opted for bilateral mastectomy if positive. In light of these data, the current study was undertaken to determine the following: rates and determinants of BRCA1/2 testing uptake in newly diagnosed high-risk BC patients; the impact of testing on surgical choices; the impact of pre-surgery testing on psychosocial well-being; and the cost effectiveness of such a program. Participants are newly diagnosed BC patients with at least a 10-20% probability of BRCA1/2 positivity. Genetic counseling and testing is performed free of charge. Thus far, 74 women have completed pre-surgery genetic counseling and testing. Unique issues arise in genetic counseling, including the assimilation of a new diagnosis of BC, issues related to prognosis versus implications of a positive test result, and unanticipated information such as ovarian cancer risks and risks to relatives. So far, of the women counseled, 60 (81%) received results before surgery, of whom 10 (17%) tested positive. Of these, 7 opted for breast conserving therapy (BCT) and 3 opted for bilateral mastectomies. Four women who received results after surgery had BCT and have not had further surgery (1 to 26 months f/u). It will be important to obtain larger numbers and follow these women long term to fully assess the impact of testing. While the current data are preliminary, the results of this study will help guide clinicians about the feasibility and implications of offering pre-surgery genetic counseling and testing. This information can be used to design genetic counseling protocols addressing the unique needs of new BC patients considering genetic testing. Supported by NCI RO1 CA/HG 74861.

A study to determine the genetic basis of uterine papillary serous adenocarcinoma. *T. Pal¹, L. Elit¹, R. Goshen², W. Chu¹, M. Mitchell¹, S.A. Narod¹.* 1) The Centre for Research in Women's Health, Sunnybrook and Women's College Health Science Centre, 790 Bay St., Toronto, Ontario; 2) Lis Maternity Hospital, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel.

Uterine papillary serous carcinoma (UPSC) shares common pathologic, genetic and clinical features with other serous cancers of mullerian origin. The most common histologic type of ovarian cancer patients with BRCA mutations is papillary serous. To the best of our knowledge, no studies have specifically investigated genetic factors in UPSC. In order to determine how frequently UPSC is due to a hereditary factor, we conducted a hospital-based study at the Princess Margaret Hospital, Toronto, Canada. Pedigrees were obtained on 79 patients diagnosed with UPSC. Family history of cancer was obtained for all 590 first-degree relatives. A DNA specimen was collected on 56 of these patients for BRCA1 and BRCA2 mutation analysis. The observed cancer rate in relatives was compared with the expected number, based on comparison with age-standardized provincial incidence rates in order to estimate relative risks. For breast cancer, there were 23 cancers observed in relatives, compared to the 15.4 cancers expected, giving a relative risk of 1.5 (95% CI: 0.9-2.2). The relative risks for endometrial, ovarian and all cancers were not elevated. Clinical review of pedigrees did not show any clear examples of the hereditary breast-ovarian cancer syndrome, and none of the 56 patients was found to carry a BRCA1 or BRCA2 mutation. Our findings suggest that there is an association between UPSC and breast cancer but this is not related to hereditary breast ovarian cancer syndrome.

Cyclin D1 Polymorphism and Age of Onset of Hereditary Malignant Melanoma. A. Platz¹, J. Hansson^{1,2}, U. Ringborg^{1,2}. 1) Cancer Centre Karolinska, Research Laboratory Radiumhemmet, Karolinska Hospital, Stockholm, Sweden; 2) Department of Oncology, Karolinska Hospital, Stockholm, Sweden.

The common A/G polymorphism in codon 224 of Cyclin D1 affects alternate splicing of exon 5. The product of the alternate spliced message lacks a sequence promoting the turnover of the protein. The relative proportion of the two possible splice products determines the level of active Cyclin D1 and variability in its expression may influence the cancer risk. We have genotyped genomic DNA from 80 individuals belonging to 80 families with Hereditary Melanoma (HMM) and from 148 normal controls for the Cyclin D1 polymorphism. The registered variant frequencies were 20% AA, 38% GG and 42% AG, in the HMM individuals, and 22% AA, 24% GG and 53% AG in the controls. We also compared genotypes with age at diagnosis for the HMM individuals and found that carriers of at least one A allele had on average a melanoma diagnosis 10 years earlier than patients homozygous for the G allele. These A allele effect may be a result of enhanced alternative splicing of the A-allele transcription product which results in a more stable Cyclin D1 lacking the PEST destruction box and is very similar to previous observations made by others on early onset of Hereditary Nonpolyposis Colorectal Cancers.

Evidence for apparent anticipation and genetic heterogeneity in familial Hodgkin's disease and non Hodgkin's lymphoma. *Y.Y. Shugart¹, K. Hemminki², P. Vaittinen³, A. Kingman⁴*. 1) Pediatrics/CIDR, Johns Hopkins University, Baltimore, MD; 2) Department of Biosciences, Karolinska Institute, Huddinge Sweden; 3) Center for Epidemiology, National Board for Health and Welfare, Stockholm, Sweden; 4) National Institute of Dental and Craniofacial Research, NIH, Bethesda, MD.

The goal of this study is to test whether there is genetic anticipation in Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL). Using the Swedish family cancer database, we identified 18 sib-pairs (2 HD and 16 NHL) and 102 parent-child pairs (13 HD, 23 HD/NHL, 8 NHL/HD and 56 NHL/NHL). The overall mean anticipation score is 27.7 years for all 102 parent-child pairs and is 13.8, 19.8, 35.8 and 28.4 years and 13.8 years for these parent-type groups, respectively. The level of anticipation is more pronounced in the NHL pairs than in HD pairs (difference=12.6 years, $p=0.0003$). These results allow us to conclude with confidence that there is apparent genetic anticipation in familial HD and NHL in the Swedish population and that HD may be associated with other types of haematopoietic malignant condition.

Interaction of CYP3A4 genotype and benign prostatic hyperplasia (BPH) with prostate cancer (PC). *C.M.*

Zeigler-Johnson, A.H. Walker, S.B. Malkowicz, T.R. Rebbeck. U. of Pennsylvania, Philadelphia, PA.

BPH is the most common benign neoplasm in aging men, but its relationship with PC etiology remains unclear. It has been hypothesized that genetic influences on testosterone metabolism may be associated with both BPH and PC etiology. For example, CYP3A4 is a member of the cytochrome P450 family that is involved in the oxidative deactivation of testosterone. A variant in the 5' regulatory region of CYP3A4 (CYP3A4-V) has been associated with advanced stage prostate cancer. In order to examine the relationship between the variant and BPH in PC etiology, we undertook a case-control study of 253 Caucasian PC cases and 428 age matched Caucasian controls. Cases were divided into two groups: (1) those with clinically detected TNM stage 1 (T1) at diagnosis; (2) those with TNM stage 2 or higher (T2+) at diagnosis. History of BPH was determined by patient report. The results of the study demonstrated that BPH was associated with PC overall (age adjusted OR=2.35, 95%CI: 1.38-3.99). CYP3A4-V was not related significantly to BPH or PC overall. The effect of CYP3A4-V was significantly different in men with and without a history of BPH. Among BPH-negative men, CYP3A4-V was significantly less common among T2+ cases than controls (age adjusted OR=0.27 95%CI: 0.09-0.79). Among BPH-positive men, CYP3A4-V was more common among T2+ cases than controls, although this result was not statistically significant due to small sample sizes (age adjusted OR=3.11, 95%CI: 0.56-17.20). No significant differences between controls and T1 cases were observed. These results suggest that CYP3A4-V and BPH may interact in the etiology of PC, and that these two factors affect the stage of PC diagnosis.

Hormonal regulation of smooth muscle proliferation in tuberous sclerosis complex. *J.J. Yu, A. Astrinidis, E.P. Henske.* Dept Medical Oncology, Fox Chase Cancer Ctr, Philadelphia, PA.

Tuberous sclerosis complex (TSC) is an autosomal dominant disease characterized by seizures, mental retardation, autism, and benign tumors in multiple organs. The smooth muscle proliferation in the lungs of TSC patients, lymphangioleiomyomatosis (LAM), appears to be driven by hormonal stimuli, but the mechanism of this is not known. The evidence for hormonal mediation of smooth muscle cell growth in LAM includes the onset of LAM almost exclusively in pre-menopausal women and the therapeutic benefit of hormonal therapy. We recently found somatic *TSC2* mutations in patients with a non-inherited form of LAM, termed sporadic LAM (PNAS 2000; 97:6085-90). We report here the establishment and analysis of primary cultures of the abnormal pulmonary smooth muscle cells from sporadic LAM patients. LAM cells in culture had positive immunoreactivity for desmin, vimentin and muscle actin. RT-PCR analysis showed transcripts of estrogen receptor (ER α) and progesterone receptor (PR). The primary cell lines were used to study the effects of estrogen and tamoxifen on the growth of LAM-derived smooth muscle cells. Estrogen at 0.01 mM stimulated cell growth to 69% and 34% of control values, and tamoxifen at 10 mM inhibited cell growth to 33% and 28% of control values, in LAM cells 635 and 650 respectively. This is, to our knowledge, the first report demonstrating an effect of steroid hormones on the proliferation of smooth muscle cells derived from LAM patients.

Pre-symptomatic mutation testing in p16-Leiden FAMMM families. *F.A. de Snoo¹, W. Bergman², A. van Haeringen¹, M.W. Zoetewij¹, H.F.A. Vasen³, N.A. Gruis², R.R. Frants⁴, M.H. Breuning¹.* 1) Department of Clinical Genetics, Leiden University Medical Center, Leiden, the Netherlands; 2) Department of Dermatology, Leiden University Medical Center, Leiden, the Netherlands; 3) Department of Gastro-enterology, Leiden University Medical Center, Leiden, the Netherlands; 4) Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands.

In a number of Dutch melanoma families a specific 19 bp deletion (founder mutation) of p16 (CDKN2A) has been found, termed p16-Leiden. P16-Leiden positive family members have a life time risk of 80% of developing pancreatic carcinoma. The latter figure, together with the exceptionally low survival rate of this disease, is a new feature in the clinical oncogenetic setting.

Since the risks in these p16-Leiden families have now been well defined, we are now able to offer pre-symptomatic mutation testing. To all p16-Leiden positive family members, skin examination on an annual basis is advised to detect melanoma development at an early stage. Simultaneously, research in the high risk group will start to determine whether pancreatic carcinoma can be detected at an early stage by intensive and regular clinical examination.

The purpose of this study is to investigate the demand and impact of pre-symptomatic testing in these well defined p16-Leiden families by using a database and questionnaires. Family members of p16-Leiden positive families will be sent a letter. This letter informs the family members that they have the possibility of predictive DNA-testing.

Preliminary results show that in the first two families approximately 30% of the people who received an informative letter made an appointment at the department of Clinical Genetics for genetic counselling.

BRCA1 and BRCA2 analysis of 268 HBOC and HBC families by DHPLC. *T. Wagner¹, R. Moeslinger¹, D. Muhr¹, D. Richards³, M. Schreiber¹, E. Fleischmann¹, Austrian Hereditary Breast and Ovarian Cancer Group¹, G. Langbauer¹, C. Zielinski², L. Jin⁵, P. Oefner⁴.* 1) Dept OB/GYN, Div Senology, Univ Vienna, Vienna, Austria; 2) Division of Oncology, Dep of Medicine!, University of Vienna; 3) Department of Genetics, Stanford University; 4) Stanford DNA Sequencing and Technology Center; 5) Human Genetics Center, University of Texas, Houston.

Using denaturing high performance liquid chromatography and sequencing, the frequencies of BRCA1 and BRCA2 mutations in 268 Austrian families with hereditary breast cancer only (HBC) or breast and ovarian cancer (HBOC) were determined. In the BRCA1 gene 27 apparently disease-associated mutations were identified in 54 (20%) families. The percentage of disease-associated mutations was highest in the 60 HBOC families (40%), ranging from 23% in families with 1 BC and 1 OC to 80% with at least 3 BC and 2 OC. The overall frequency of BRCA1 mutations in the 193 HBC families was 14%, and as high as 50% in families with at least 4 BC <60 years. Nine founder mutations were detected that accounted for two thirds of all BRCA1 mutations. The two most frequently identified mutations were 1806CtoT (7 times) and 300TtoG (6 times). Additionally 10 unclassified variants were detected in the coding region of BRCA1. In the BRCA2 gene 10 apparently disease-associated mutations were identified in 17 (6%) families. One founder mutation accounted for 47% of all BRCA2 families in Austria. The percentage of disease-associated mutations was highest in families with male and female breast cancer (29%). In HBC families 5% could be explained by BRCA2 mutations, ranging from 7% in families with 3 BC cases to 25% with 4 BC < 60 years. In HBOC families only 8% BRCA2 mutations were detected. In the coding region of BRCA2 6 unclassified variants were detected. In conclusion 26% of families with HBC or HBOC can be explained by disease associated BRCA1 or BRCA2 mutations.

Further characterization of MTR1, a novel gene from the BWS-WT2 critical region on chromosome 11p15.5 and its murine homolog. *D. Prawitt¹, T. Enklaar¹, L. Brixel¹, M. Thesker¹, A. Langer¹, C. Spangenberg¹, S. Fees¹, M. Higgins², B. Weissman³, B.U. Zabel¹.* 1) Molecular Genetics Lab, Children's Hosp/ Univ Mainz, Mainz, Germany; 2) Roswell Park Cancer Center, Buffalo, NY, USA; 3) Lineberger Comprehensive Cancer Center, Univ. of North Carolina, NC, USA.

Beckwith-Wiedemann syndrome (BWS) and a variety of mostly embryonal neoplasias (e.g. Wilms'tumors (WT), rhabdoid tumors and rhabdomyosarcomas) display genetic linkage to the human chromosomal region 11p15.5. By using resources of EST- and genome sequencing projects we isolated a novel transcript which shows high homologies to the transient receptor potential protein (trp) gene family. The highest homologies are observed with the human TRPC7 and with MLSN1 (a trp-related protein whose transcript is downregulated in metastatic melanomas). The novel gene was therefore named MTR1 (= MLSN1 and TRP related gene). This gene family includes mediators of ion influx into cells after stimulation with growth hormones (e.g. IGF1). Other genes related to and interacting with the trp-family encompass the grc-gene which codes for a growth factor regulated channel protein and PKD1/PKD2, integral membrane proteins, involved in polycystic kidney disease. Increased amounts of MTR1 mRNA are present in a significant proportion of WTs and rhabdomyosarcomas. We also found that MTR1 maps near a translocation-breakpoint in the rhabdoid tumor cell line TM87-16. To elucidate the function and implication of MTR1 in BWS and 11p15.5 associated tumors, we also isolated and characterized the murine homolog, which maps to the corresponding region on distal mouse chromosome 7. RT-PCR analysis of somatic cell hybrids harboring a single human chromosome 11 demonstrated exclusive expression of MTR1 in cell lines carrying a paternal chromosome 11, indicating allele-specific inactivation of the maternal copy by genomic imprinting. In contrast to this, the murine gene displays biallelic expression in all tissues and developmental stages tested. We are currently looking for mutations in BWS- and tumor materials. Further functional studies include tumor suppression experiments in G401/RD cells and search for interacting partners with a yeast two-hybrid assay.

Mutations in the SDHD gene are not only involved in hereditary paragangliomas in the head and neck region, but also in adrenal and extra-adrenal pheochromocytomas. *P.E. Taschner¹, P.B. Douwes Dekker^{2,3}, J.C. Jansen², B.E. Baysal⁴, A. Bosch¹, I.H. van Minderhout¹, E.H. Rosenberg¹, A.G. van der Mey², A.H. Bröcker-Vriends¹, G.J. van Ommen¹, C.J. Cornelisse³, P. Devilee^{1,3}.* 1) Human and Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands; 2) Otorhinolaryngology, Leiden University Medical Center, Leiden,; 3) Pathology, Leiden University Medical Center, Leiden,; 4) Psychiatry, The University of Pittsburgh Medical Center. Pittsburgh, PA.

Hereditary paragangliomas or glomus tumors are usually benign slow-growing tumors in the head and neck region. Occasionally, paragangliomas outside the head and neck region, such as adrenal and extra-adrenal pheochromocytomas, have been reported in patients with head and neck paragangliomas. The inheritance pattern of hereditary paraganglioma is autosomal dominant with imprinting. The occurrence of hereditary paragangliomas has been linked to two different loci, PGL1 on chromosome 11q22-q23 and PGL2 on 11q13. Most families with hereditary paraganglioma are linked to the PGL1 locus. Recently, we have identified the SDHD gene encoding subunit D of the mitochondrial respiratory chain complex II as the PGL1 gene. Two founder mutations, D92Y and L139P, account for the majority of the Dutch paraganglioma families. These two mutations were also detected among 40% of the isolated patients. Ten of these had multiple paragangliomas, and in 8 out of these 10, SDHD germline mutations were found, indicating that multicentricity is a strong predictive factor for the hereditary nature of the disorder in isolated patients. In head and neck paragangliomas from mutation-carrying patients, loss of the maternally derived wildtype SDHD allele has been observed, indicating that SDHD functions as a tumor suppressor gene. We demonstrate that loss of the maternally derived wildtype SDHD allele also occurs in adrenal and extra-adrenal pheochromocytomas in SDHD mutation carriers. This indicates that SDHD mutations are not only involved in hereditary head and neck paragangliomas, but also in adrenal and extra-adrenal pheochromocytomas. This research is supported by the Dutch Cancer Society (KWF 98-1721).

Germ-line BRCA1 variant R1699W: A BRCT domain marker of site-specific hereditary cancer.. *J.L. Scalia¹, T.S. Frank², P.L. Weinstein¹, I. Tepler¹*. 1) Cancer Risk and Prevention Program, The Stamford Hospital, Stamford, CT; 2) Myriad Genetic Laboratories Inc, Salt Lake City, UT.

BRCA1 and BRCA2 germ-line mutations account for an estimated 80-90% of hereditary breast and ovarian cancer families and are present in the majority of familial epithelial ovarian carcinomas. Several recent studies have reported the nuclear localization of BRCA1 with functional evidence of DNA repair and transcriptional activation. Protein interaction with the BRCA1 C-terminal (BRCT) domain may partially account for the mediating role of transcriptional regulation as a response to DNA damage. To investigate whether the presence of a BRCT domain variant directly correlates to specific clinical expression, we analyzed the presence of the BRCA1 variant R1699W in our family TSH102-99. The variant results in the substitution of tryptophan for arginine at amino acid position 1699 of the BRCA1 protein. This exon18 missense mutation is positioned in the conserved residue of the first tandem BRCT domain. Across three generations, and four degrees of relatedness, every woman diagnosed with ovarian cancer in this family is a demonstrated or obligate carrier of this rare BRCA1 variant. There was no breast cancer in the family. This case study indicates a BRCT variant (R1699W) as a marker, and highly suggestive cause, of site-specific hereditary cancer.

Protocols for the investigation of large, genomic alterations in the DNA mismatch repair genes MLH1, MSH2, and MSH6 by southern blot analysis. *R.E. Pyatt¹, M. Sedra¹, R.W. Schafer¹, A. de la Chapelle², T.W. Prior¹.* 1) Dept. of Pathology, Ohio State University, Columbus, OH; 2) Division of Human Cancer Genetics, Ohio State University, Columbus, OH.

Hereditary nonpolyposis colorectal cancer (HNPCC) is one of the most common inherited forms of colorectal (CRC) cancer and is characterized by an early age of onset, microsatellite instability (MSI), and an autosomal dominant mode of inheritance. Tumors in the HNPCC syndrome are primarily caused by inherited mutations in three of the six DNA mismatch repair (MMR) genes: MLH1, MSH2, and MSH6. While extensive research has been done identifying small types of mutations in these genes, there is little information in the literature examining the presence of large deletions and other rearrangements in them. In this study, we report the development of southern blot protocols for the complete analysis of the DNA MMR genes MLH1, MSH2, and MSH6 for large, genomic alterations. Hind III and NSI 1 restriction enzyme digestion patterns were defined using lymphocyte DNA from 150 normal controls hybridized with two cDNA probes spanning MSH6, two cDNA probes spanning MSH2, and a single MLH1 CDNA probe encompassing the entire gene. For these enzymes, no polymorphic variations in the restriction patterns were found in the control population examined. Using these protocols, lymphocyte DNA from 10 individuals with MSI+ CRC tumors and 20 individuals with MSI- CRC tumors was evaluated for the presence of junction bands and altered band intensity. In an individual with MSI+ CRC, a junction band was identified using a cDNA probe corresponding to exons 1-7 of the MSH2 gene. This junction band was further shown to be maternally inherited and, through both long PCR and exon specific quantitative PCR reactions, the exact nature of these findings are being elucidated in both individuals. The southern protocols outlined here allow for a comprehensive analysis of large, genomic alterations in the DNA MMR genes.

***SDHD* gene testing in "sporadic" paraganglioma indicates a high proportion of unrecognized heritable cases.**

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Non-chromaffin paragangliomas (PGLs) are slow-growing tumors of the head and neck that cause substantial morbidity due to cranial nerve impairment. In PGL families, there is considerable potential to avert cranial nerve damage by prospective MRI. Hereditary PGL shows genetic heterogeneity with at least 3 loci. PGL1 is the succinate-ubiquinone oxidoreductase subunit D gene (*SDHD*) recently identified by our group. Inheritance is autosomal dominant with maternal imprinting in PGL1 and PGL2 families but not PGL3. The heritable proportion is widely estimated at 7-50% and is probably obscured in part by imprinting.

We assessed the heritable proportion of PGL by SSCP analysis of the 4 *SDHD* exons. We analyzed cheek swab DNA from 39 individuals with PGL ascertained in the U.S. without respect to family history or tumor multifocality. Sixteen samples showed aberrant SSCP bands. Of 8 subjects with heritable features, 7 had aberrant bands. Of the 31 apparently sporadic cases, 9 had aberrant SSCP bands and another 3 showed SSCP band shifts of uncertain significance. An aberrant band was seen in a woman affected at age 35, suggesting young age of onset alone may help to identify some heritable cases. However an aberrant band was also seen in a woman with the classical sporadic presentation, PGL diagnosis at age 82.

These data suggest that about a third of patients with a single PGL tumor and no PGL family history (as elicited by a genetic counselor) have germline *SDHD* mutations. If confirmed by DNA sequencing (in progress), this indicates that heritable PGL cannot be adequately discerned by clinical history alone. *SDHD* gene testing of apparently sporadic cases (analogous to *RET* testing for medullary thyroid cancer) may help to identify a significant proportion of people at risk for PGL tumors.

Prevalence of germline MMR gene mutations in French kindreds with HNPCC or aggregation of colorectal cancers. *Q. Wang¹, C. Lasset², F. Desseigne³, E. Ruano¹, C. Navarro¹, G. Montmain¹, A. Puisieux¹.* 1) Unité d'Oncologie Moléculaire; 2) Département de Santé public; 3) Département de Médecine, Centre Léon Bérard, Lyon, France.

Hereditary nonpolyposis colon cancer (HNPCC) syndrome is an autosomal dominantly inherited predisposition for early onset of colorectal cancer. It accounts for about 10% of total colorectal malignancies. This syndrome results from germline mutations of one of the MMR genes: hMLH1, hMSH2, hPMS1, hPMS2 and hMSH6. Identification of germline mutations of MMR genes has an important clinical interest in the management of HNPCC families. The aim of this study was to evaluate the involvement of MMR gene mutations in different groups of families, to study genotype and phenotype correlation, and to estimate *de novo* mutation frequency, in order to establish appropriate criteria for the selection of the families for mutation screening. We analyzed the status of hMLH1 and hMSH2 genes in 130 french patients by using a combined DNA- and RNA- based strategy. A total of 53 alterations were identified. The majority of the mutations were found in typical HNPCC families (more than 60%), whereas about 30% were identified in atypical families missing 1 or 2 Amsterdam criteria. In the absense of family history, mutations were mainly found in patients affected with multiple primary cancers. Most of the alterations were nonsense mutations and small insertions/deletions leading to the generation of prematured stop-codon. However, missense mutations were found in 19 patients, mainly in the hMLH1 gene. Our study shows 1) the use of sensitive technologies for the detection of the mutations; 2) a very low *de novo* mutation frequency in MMR genes; and 3), the requirement of appropriate criteria which should include multiple primary cancers among the guidelines for referring patients for genetic testing .

Tumor suppressor function of the BRCA1 gene has a low threshold for mutation. *S.R. Svojanovsky, P.K. Rogan.*
Section Medical Genetics & Molecular Medicine, Children's Mercy Hosp, Univ Missouri-Kansas City School of Medicine.

Mutations in the *BRCA1* gene are penetrant, presumably because the tumor suppressor activity is highly susceptible to mutation. The susceptibility may be related to extensive alternative splicing in this gene, which diminishes the amount of full-length transcript produced in normal tissue. Tumor suppressor activity has been mapped to the C-terminal domain of the protein. Because of alternative splicing, we considered the possibility that tumor suppression by this gene might be susceptible to mutations that reduced, but did not abolish production of the full-length transcript. BRCA1 splice site variants reported in individuals with a history of breast cancer (n=45; Breast Cancer Information Core, NHGRI, NIH) were evaluated by individual information (R_i) analysis. Comparison of R_i values of normal and variant splice site sequences can predict changes in levels of normally spliced mRNA that result from nucleotide substitutions. The majority of BRCA1 mutations were severe (n=33, $DR_i > 6.6$ bits, ie. >100 fold decrease in splice site recognition), however 4 mutations were predicted only to diminish the levels of normally spliced mRNA (IVS13+6 T>C, IVS16+6 T>C, IVS22+5 G>A, and IVS23-10 C>A; between 3 and 11 fold decrease). Eight variants did not alter R_i values of the adjacent splice site and may be polymorphisms.

Nine N-terminal splicing mutations, which were predicted to be severe, remained in frame, suggesting that coding sequence deletions upstream of the C-terminal domain can also diminish tumor suppression. Surprisingly, IVS13+6 T>C, a mild mutation which also occurs upstream of the C-terminal tumor suppressor domain and maintains the reading frame, was found in a breast cancer family. Failure of this and the other leaky splicing mutations to suppress breast tumors is consistent with the possibility that a high level of normal *BRCA1* mRNA may be required to suppress tumor formation.

Mutational analyses of BRCA1 and BRCA2 in Ashkenazi and non-Ashkenazi Jewish women with familial breast and ovarian cancer. *R. Shiri-Sverdlov¹, P. Oefner³, R. Gershoni-Baruch², L. Green¹, RMW. Hofstra⁵, T. Wagner⁴, E. Friedman¹.* 1) Oncogenetics Unit, Sheba Medical Center, Tel-Hashomer, Israel; 2) The Genetics Institute, Rambam Medical Center, Haifa; 3) The Department of Genetics, Stanford DNA sequencing and technology Center, Palo Alto CA; 4) Department of Obstetrics and Gynecology, Division of Senology, University of Vienna, Vienna, Austria; 5) The Department of Medical Genetics, Groningen, Netherlands.

ABSTRACT In Ashkenazi (East European) Jews, three predominant mutations in BRCA1 (185delAG and 5382insC) and BRCA2 (6174delT) account for the majority of germline mutations in high risk breast and/or ovarian cancer families. Among non-Ashkenazi Jews, the 185delAG, Tyr978X, and a handful of "private" mutations have anecdotally been reported within both genes. In this study we attempted to determine the spectrum of BRCA1 and BRCA2 mutations in high risk Jewish individuals, non-carriers of any of the predominant Jewish mutations. We employed multiplex PCR and denaturing gradient gel electrophoresis analysis for BRCA2, and combined denaturing high pressure liquid chromatography and protein truncation test (PTT) for BRCA1, complemented by DNA sequencing. We screened 47 high risk Jewish individuals, 26 Ashkenazis and 21 non-Ashkenazis. Overall, 13 sequence alterations in BRCA1 and 8 in BRCA2 were detected: 9 neutral polymorphisms and 12 missense mutations, including 5 novel ones. The novel missense mutations did not cosegregate with disease in BRCA1 and were detected at rates of 6.25% to 52.5%, in the general population for BRCA2. Our findings suggest that except for the predominant mutations in BRCA1 and BRCA2 in Jewish individuals, there are only a handful of pathogenic mutations within these genes. It may imply novel genes may underlie inherited susceptibility to breast/ovarian cancer in Jewish individuals.

A diagnostic screening test for tumor microsatellite instability (MSI) using fluorescent multiplex PCR and automated fragment analysis. *M. Rose*^{1,2}, *T. Selander*², *M. Redston*^{1,2,3}, *S. Gallinger*^{1,2,3}, *B. Bapat*^{1,2,3}. 1) Center for Cancer Genetics, Samuel Lunenfeld Research Institute; 2) Departments of Pathology and Laboratory Medicine, and Surgery; Mount Sinai Hospital; 3) University of Toronto, Toronto, Ontario.

Microsatellite instability (MSI) is defined as the occurrence of novel alleles in tumor DNA with a frequency of at least 40% among microsatellite loci examined. Defects in mismatch repair system cause MSI, which plays an important role in the development of tumors. MSI is a hallmark of a subset of sporadic colorectal and HNPCC-associated tumors. Detection of tumors with MSI has important prognostic and treatment implications for patients. We describe the development of an efficient diagnostic test for screening of tumor MSI based on fluorescent multiplex PCR system and automated fragment analysis. The assay consists of a panel of 11 microsatellite loci including those loci (BAT25, BAT26, D2S123, D5S346 and D17S250) recommended by the National Cancer Institute (1). The microsatellite panel includes one tetranucleotide, six dinucleotide and four mononucleotide loci. DNA extracted from paraffin embedded tissue (25 ng) is amplified in two multiplex PCR reactions. The fluorescent labelled PCR amplicons (size range 65-230 bp) are analyzed using ABI377 GeneScan and Genotyper software. This diagnostic assay was validated by analyzing ten colorectal cancer cases by both fluorescent multiplex PCR and conventional radioactive labelled PCR and gel electrophoresis. The assay sensitivity was determined by MSI analysis of tumor DNA serially diluted with matched normal DNA, and was found to range from 10% for mononucleotide loci to 40% for dinucleotide loci. Overall, this diagnostic assay offers a fast, sensitive and cost-effective method of MSI detection and is most suitable for high throughput screening for mismatch-repair deficient tumors. (1) Boland R. et al. 1997; Cancer Res. 58:5248-57.

Insulin like growth factor I (IGF-I) polymorphism and breast cancer risk in Jewish women. *Y. Patael-Karasik¹, A. Figer², R. Gershoni-Baruch³, A. Chetrit⁴, M.Z. Papa⁵, R. Bruchim-Bar-Sade¹, E. Friedman¹.* 1) Oncogenetics Unit, Sheba Medical Center, Tel-Hashomer, Israel; 2) Oncology Institute, Rabin Medical Center, Petach Tikvah; 3) The Genetics Institute, Rambam Medical Center, Haifa; 4) the Department of Clinical Epidemiology, Sheba Medical Center, Tel-Hashomer; 5) The Department of Oncological Surgery, Sheba Medical Center, Tel-Hashomer.

Background: Genes that confer mild or moderate susceptibility to breast cancer, may be involved in the pathogenesis of sporadic breast cancer, and modify the phenotypic expression of mutant BRCA1/BRCA2 alleles. An attractive candidate is the Insulin-like growth factor I (IGF-I), a known mitogen to mammary ductal cells in vivo and in vitro, whose serum levels were reportedly elevated in breast cancer patients. Methods: We analyzed allele size distribution of the polymorphic CA repeat upstream of the IGF-I gene in 412 Jewish Israeli women: 268 women with breast cancer (212 sporadic and 56 carriers of either a BRCA1 or BRCA2 mutations), and 144 controls. The methodology employed included radioactive PCR amplification of the relevant genomic region, gel size separation, and autoradiography, followed by statistical analyses of allele size distribution within the different analyzed groups. Results: Among women with breast cancer, with or without BRCA germline mutations, the 196 and 198 basepair (bp) alleles were present in 4.7% (25/536 alleles), compared with 9% (26/288) controls ($p=0.02$). This difference was more pronounced and significant in the non-Ashkenazi population. Conversely, the smaller size allele (176 bp) was only present in the breast cancer group (3/536- 0.6%), and in none of the controls. Conclusion: We conclude that the IGF -I polymorphism may serve as a marker for breast cancer risk in the general Jewish population, in particular non-Ashkenazi Jews, but an extension and confirmation of these preliminary data is needed.

Developing a regional cancer genetics network: Linking a center of excellence to the community laboratory. *J.N.*

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Barriers to the application of genetic cancer risk assessment (GCRA) protocols include limited access to counseling and education services that are equipped to handle the complex and rapidly evolving technological, and ethical issues.

Objective: Explore mechanisms for technology transfer of advanced GCRA protocols, research and education to community physicians outside the primary service area of a multidisciplinary clinical cancer genetics program at a comprehensive cancer center.

Methods: Identify champions in community hospitals to sponsor educational programs and provide on-site coordination of care; provide experienced personnel, GCRA protocols and opportunities for participation in clinical research; deliver CME-qualified cancer genetics education to clinicians and in public forums; use informatics to augment teaching and catalogue resources.

Results: The City of Hope (COH) *Cancer Screening & Prevention Program Network* (CSPPN) reaches the Santa Barbara, Orange and Los Angeles counties of Southern California (5 sites), and a new satellite site in Phoenix, AZ. Coordination of CME events and clinic opening increased both community physician knowledge and appropriate referrals (550 families to date). Two sites employ a team approach, where the oncogeneticist and the cancer risk counselor provide comprehensive services together. A solo cancer risk counselor provides initial counseling at one outlying site; these patients may travel to the COH campus to see the team for discussion of complex management issues and/or disclosure of genetic test results. Cases are reviewed in a weekly multidisciplinary conference. The CSPPN yields clinical and biologic resources for genetic epidemiology and outcomes research through an IRB-approved confidential registry. This regional cancer genetics network represents a community laboratory for the development of innovative mechanisms for delivery of GCRA protocols.

The Homeobox gene *CDX2* in colorectal carcinomas; a genetic analysis. *S. Sivagnanasundaram*¹, *I. Islam*¹, *I. Talbot*², *F. Drummond*¹, *J.R.F. Walters*³, *Y.H. Edwards*¹. 1) MRC Human Biochemical Genetics Unit, Department of Biology, University College London, London; 2) Academic Department of Pathology, St Mark's Hospital, Northwick Park, London; 3) Gastroenterology Section, Division of Medicine, Imperial College School of Medicine, Hammersmith Hospital, London.

Accumulation of mutations in tumour suppressor genes and oncogenes has been proposed to underlie the initiation and progression of sporadic colorectal cancer (CRC). Evidence is accumulating to suggest that the *caudal* homeobox gene *CDX2* is implicated in the pathogenesis of CRC. The *CDX2* transcription factor is expressed in intestinal epithelium and is markedly down-regulated in colon tumours. Furthermore, *Cdx2* heterozygous null mice develop multiple intestinal tumours. In this present study, we have investigated *CDX2* as a potential candidate gene for sporadic CRC by a thorough search of all exons and exon/intron boundaries for DNA polymorphisms and rare variants in a panel of 51 CRC tumour/normal pairs. Six polymorphisms were identified and the haplotypes determined. In addition two rare variants were found, one of which was only identified in DNA from a CRC case. Loss of heterozygosity was observed in 3 out of 28 informative CRC cases. A possible association between particular haplotypes and tumour progression was also suggested by the data. In addition a preliminary analysis of the relative expression of *CDX2* alleles in tumour/normal tissue suggested some variation in the levels, however further analysis is required before any conclusions can be drawn. While *CDX2* mutations predisposing to sporadic CRC have not been identified, this study has established that loss of *CDX2* contributes towards the progression of some sporadic CRC tumours.

MLH1 and MSH2 mutation identification from unselected patients with colorectal cancer is facilitated by the combined analysis of microsatellite instability and immunohistochemistry. *C. Verellen-Dumoulin¹, V. van*

Scherpenzeel Thim¹, A. Kartheuser², M. Smaers¹, P. Camby³, R. Detry², C. Sempoux³, K. Dahan¹. 1) Ctr for Human Genetics, UCL, Brussels, Belgium; 2) Dpt of Surgery, UCL, Brussels; 3) Dpt of Pathology, UCL, Brussels.

Colorectal cancer (CRC) morbidity and mortality can be dramatically reduced by appropriate management of patients with hereditary non polyposis colorectal cancer (HNPCC) and by surveillance of at-risk individuals. The aim is to find a approach to identify HNPCC patients on a large scale in a sensitive and efficient manner. **PATIENTS AND METHODS:** From 1998 until 2000, CRC specimens from consecutively new patients were studied for microsatellite instability (MSI) and expression of MLH1 and MSH2 proteins. HNPCC germline mutation search was carried out for MSI(+) individuals according to the immunochemistry results. **RESULTS:** Among 225 CRC, 45 (20%) were MSI high associated with lack of immunostaining in 32: 25 for *MLH1* and 7 for *MSH2*. Among these, until now, we have found 8 (3.5 % of all CRC) disease-causing germline mutations, 5 in *MLH1* and 3 in *MSH2*. Seven are new ones. The functional consequences result from 2 frameshifts leading to stop codons, 2 splice defects and 4 missenses. Among these 8 patients, 4 were older than 50 years, 7 had at least 1 first-degree relative with CRC or endometrial cancer and 1 had a Turcot syndrome. **CONCLUSION:** A systematic MSI study associated to immunohistochemistry is an efficient strategy to identify HNPCC patients from an unselected cohort of patients with CRC. This approach allowed us to make the diagnosis of HNPCC in 8 as yet unrecognized patients and to start a predictive HNPCC screening in their family.

Cellular localization of nonfunctional BLM protein in cells from persons with Bloom's syndrome (BS) who have inherited missense mutations. *M.M. Sanz¹, N.A. Ellis², J. German¹*. 1) Laboratory of Human Genetics, Dept. of Pediatrics, Cornell Univ. Medical College; 2) Laboratory of Cancer Susceptibility, Dept. of Human Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY.

In cells from normal persons examined by immunofluorescence microscopy employing antiBLM antibody, BLM is detectable in the nucleus of all except very early G1 cells, distributed fairly diffusely and usually also concentrated focally in tiny speckles, large dots, and large patches. Earlier we demonstrated a total lack of BLM protein in cells from persons with BS caused by either homozygosity or compound heterozygosity for protein-truncating mutations at BLM [AJHG 65(Suppl.):A320,1999]. Among the 65 mutations in BLM detected in persons followed in the Bloom's Syndrome Registry, protein-truncating mutations are the commonest class, but 10 are missense mutations. In the analysis of three of the persons with BS who inherited either one or two missense mutations, BLM has been found in what on preliminary examination appears to be a normal distribution. The present report is of (i) the amount and cellular distribution of BLM encoded by the various BS-causing missense mutations detected comparing homozygotes, genetic compounds with two different missense mutations, and genetic compounds with one missense and one truncating mutation; and (ii) significant phenotype correlations between the mutation, the nuclear distribution of BLM, the BS clinical and cytogenetic (SCE) phenotypes, and the development of cancers.

***De novo BRCA2* mutation in a patient with early-onset breast cancer.** M. Robson, L. Scheuer, R. Baum, J. Hull, D. McDermott, H. Pierce, K. Nafa, N. Ellis, K. Offit. Dept Human Genetics, Mem Sloan-Kettering Cancer Ctr, New York, NY.

To date there is no published report of a *de novo BRCA2* mutation. We report a patient with a *de novo BRCA2* mutation, 7260insA, previously undescribed in the Breast Information Core database. The proband was a 41 year old female diagnosed with synchronous bilateral breast cancer at age 35. Family history was significant for a primary gastric cancer diagnosed in her brother at age 47, metachronous diagnoses of colorectal and gastric cancer in her father at ages 57 and 62, and a diagnosis of pancreatic cancer in a paternal grandfather in his sixties. All affected family members were deceased. Of note, the patient had 10 other siblings (5 brothers and 5 sisters), none of whom had cancer diagnoses. Following informed consent, sequencing of *BRCA1* and *BRCA2* was performed on the proband's leukocyte-derived DNA, which revealed a frameshift mutation, *BRCA2* 7260insA, which causes premature termination at codon 2359. The proband's mother and five sisters all underwent testing for the 7260insA mutation by direct, single amplicon sequencing. The mutation was not present in any of their samples. In an attempt to detect the 7260insA mutation in the father, who is deceased, a colon tumor block was acquired. After extraction of DNA from the paraffin-embedded tissue, DNA sequencing analysis was performed, and the mutation was not present. DNA genotyping analysis at informative microsatellite loci supported the paternity of the father. The frequency of *de novo* mutations of *BRCA1* and *BRCA2* has not yet been determined. Given the large size of *BRCA2* (27 exons distributed over approximately 70kb of genomic DNA) and evidence of mutational "hot spots," it is not surprising that *de novo* mutations exist. Failure to consider the occurrence of *de novo* mutations will result in an underestimate of penetrance estimates derived from models that are based on genotyping of probands with incomplete or no genotyping of parents. The existence of *de novo* mutations must be considered in counseling individuals with early onset breast and/or ovarian cancer.

The APC/b-Catenin/Tcf-4 (ACT) signaling pathway, survivin expression, and apoptosis in normal and malignant human colonic epithelial cells. *T. Zhang, T. Otevrel, Z.Q. Gao, Z.P. Gao, A. Kairo, S. Burkholder, S. Ehrlich, B.M. Boman.* Division of Medical Genetics, Thomas Jefferson University, Philadelphia, PA.

Survivin is an anti-apoptotic gene that is expressed in cells during embryological development or after malignant transformation, but its expression is suppressed in differentiated cells. Indeed, we have shown in normal human colonic crypts that survivin is preferentially expressed in the proliferative compartment as compared to the compartment containing differentiated colonocytes. The ability of survivin to inhibit apoptosis is thought to be important in tumorigenesis. However, cellular factors that normally regulate survivin gene expression and that are involved in dysregulation of its expression during cancer development are unknown. Because ACT signaling modulates transcription of a number of target genes and APC mutation causes development of colorectal cancer (CRC), we investigated a possible role of ACT signaling in the regulation of survivin gene expression in a CRC cell line (HT-29). In one experimental approach, we used a recombinant HT-29 cell line, HT29-APC, which contains a wild type (wt) APC construct with a zinc inducible promoter. Zinc induction of wt-APC causes apoptosis in this recombinant cell line. Upon zinc induction, a significant decrease in the level of survivin expression was observed using immunocytochemistry and RT-PCR as well as a marked increase in cell death. In another experimental approach, we attempted to inhibit tcf-4 mediated activation of gene transcription by using a dominant negative tcf-4 gene construct. In an initial pilot study, this construct was transiently transfected into HT-29 cells. We observed, using RT-PCR, that survivin expression decreased within 24 hours and the suppression of survivin expression correlated with the amount of tcf-4 construct transfected. These two experimental findings are consistent with each other and suggest a tumorigenic mechanism whereby APC mutation and tcf-4 activation leads to constitutive expression of survivin, thereby preventing apoptosis and contributing to the development of malignant colon cells.

Two separate regions of functional tumor suppressor activity on chromosome 10p involved in prostate cancer.

P.E. Wong, M.M. Lovell, A.M. Killary. Dept Pathology & Lab Med, Univ Texas MD Anderson CA Ctr, Houston, TX.

A major goal of our laboratory's research is to use a functional genomic approach to define and isolate tumor suppressor genes involved in prostatic adenocarcinoma. Previously, we have defined a novel tumor suppressor locus *PAC-1* within chromosome 10p. Introduction of the short arm of chromosome 10 into a prostatic adenocarcinoma cell line PC-3H resulted in dramatic tumor suppression and restoration of a programmed cell death pathway. To functionally dissect the region within 10p containing *PAC-1*, we developed the novel strategy of serial microcell fusion, a technology that would allow the transfer of defined fragments of chromosome 10p into PC-3H and the rapid *in vivo* assay for functional tumor suppressor activity. Serial microcell fusion was used to transfer defined 10p fragments into a mouse A9 fibrosarcoma cell line. Once characterized by FISH and microsatellite analyses, the 10p fragments were subsequently transferred into PC-3H to generate a panel of microcell hybrid clones containing overlapping deletions of chromosome 10p. *In vivo* and microsatellite analyses of these PC hybrids identified four clones that limited a region of functional tumor suppressor activity to 10p11 and flanking a region of LOH demonstrated in prostate cancer. In addition, these clones further limited a second unique region within 10p15 distinct from two regions identified by LOH in different histologic neoplasms. These studies demonstrate the utility of this approach as a powerful tool to limit regions of functional tumor suppressor activity. Furthermore, these data used in conjunction with data generated by the Human Genome Project will lend a focused approach to identify candidate tumor suppressor genes involved in prostate cancer.

Risk-reducing surgery outcomes among a series of *BRCA* heterozygotes. *L.M. Scheuer¹, M. Robson^{1,3}, R. Baum¹, M. Capasso¹, C. Duteau-Buck¹, J. Hull¹, B. Kelly¹, D. McDermott¹, H. Pierce¹, M. Pinto¹, C. Schulz¹, R. Barakat², P. Borgen², C. Hudis³, L. Norton³, K. Offit^{1,3}.* 1) Dept. of Human Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY; 2) Dept. of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY; 3) Dept. of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY.

Women with mutations in *BRCA1* and *BRCA2* are at increased risk to develop breast and ovarian cancers. Some of these women decide to undergo risk-reducing salpingo-oophorectomy (RRSO) and/or risk reducing mastectomy (RRM). We have obtained follow-up data from 2 months to 5 years after genetic testing on 169 women with pathogenic *BRCA* mutations. Eighty-five of the 169 women (50.3%) underwent RRSO, the vast majority (74/85 or 87.1%) after receiving genetic test results. Of the remaining women, 16 underwent oophorectomy for treatment of ovarian/fallopian tube/ or peritoneal cancers (14 diagnosed clinically and 2 diagnosed after surgery for an abnormal screening ultrasound), 9 for other gynecologic reasons, and 3 for abnormal screening findings proven not to be malignant at surgery. Fifty-six *BRCA* heterozygotes had their ovaries intact at follow-up. Four of the 85 women (4.7%) who underwent RRSO were diagnosed with clinically occult cancers. This includes 2 women diagnosed with ovarian cancers at ages 38 and 52, 1 with a fallopian tube cancer at age 47, and 1 with pathology pending at age 48. The mean age at RRSO among those without occult cancers was 46.4 years.

Forty-two women (24.9%) underwent RRM, including 31 with and 11 without breast cancer. Of 41 women who underwent RRM, 25 (61.0%) did so after receiving genetic test results and 16 (39.0%) prior to receiving results. The mean age at RRM was 41.7 years. The majority, 35/41 (85.4%) of women underwent reconstruction. One woman (2.4%) was diagnosed with an unsuspected intraductal cancer at age 50.

Among our series of *BRCA* heterozygotes, a significant proportion have chosen to undergo risk-reducing surgeries. Four women (4.7%) who underwent RRSO and 1 (2.4%) who underwent RRM were detected to have early-stage cancers at the time of these procedures.

High-resolution analysis of gene copy number changes across the genome in hundreds of tumors: CGH on cDNA microarrays followed by FISH analysis on tissue microarrays identify genes involved in DNA amplifications at 17q23 in breast cancer. *A.H. Kallioniemi¹, O.M. Monni¹, J. Kononen¹, M. Barlund², S. Mousses¹, J.D. Weaver¹, M. Bittner¹, Y. Chen¹, J. Torhorst³, G. Sauter³, O-P. Kallioniemi¹.* 1) Cancer Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Institute of Medical Technology, University of Tampere and Tampere University Hospital, Finland; 3) Institute of Pathology, University of Basel, Switzerland.

Due to the limited resolution of classical chromosomal CGH, microarray-based CGH methods have been developed for high-resolution analysis of chromosomal changes in cancer and other diseases (Pinkel, 1998, Pollack et al. 1999, and Heiskanen et al. 2000). However, informative breakpoints for copy number changes are often rare, requiring analysis of hundreds of specimens, which is not easy to achieve with such DNA chip based approaches. Here, we present a novel strategy for genome-wide high-resolution mapping of copy number changes in hundreds of tumors based on combination of CGH microarray analysis with hundreds of mapped cDNA clones, followed by assembly of a high-resolution panel of BAC probes for tissue microarray-based FISH analysis on hundreds of tumors. In order to identify genes involved in DNA amplifications taking place along 17q in breast cancer, we constructed a chromosome-specific cDNA microarray, covering RH-mapped known genes and Unigenes. Breast cancer cell lines with high-level DNA amplifications at 17q23 by chromosomal CGH revealed a region of high-level copy number at cR 372 to 385. In order to explore this site in more detail, a set of 10 BAC probes for this interval was generated, and applied in FISH analysis of 372 primary breast cancers placed in a tissue microarray. This analysis was able to narrow down the region of involvement, from which a novel overexpressed, and rearranged MB-17A gene was found. In summary, this study illustrates a powerful high-throughput CGH-microarray and tissue microarray-based strategy to utilize the genomic sequence and resources to identify target genes for chromosomal rearrangements in cancer.

Comparison of Neural Network Modeling and Logistic Regression for Predicting *BRCA1* and *BRCA2* Mutations in Women with Ovarian Cancer. *M.-P. Dube¹, J.-S. Brunet¹, R. Moslehi^{1, 2}, S.A. Narod¹*. 1) Public Health Sciences, University of Toronto, Toronto, Ontario, Canada; 2) Department of Medical Genetics, University of British Columbia, British Columbia, Canada.

The goal of this study is to develop an accurate model for predicting the presence of a *BRCA1* and *BRCA2* founder mutation among Jewish women with ovarian cancer. We performed genetic testing for two mutations in *BRCA1* (185delAG and 5382insC) and one mutation in *BRCA2* (6174delT) in 208 Jewish women with ovarian cancer. A total of 86 mutations were identified. We then designed prediction models for the presence of a mutation, which incorporate information on the age of onset of the ovarian cancer, the histology, stage and grade of the tumor, and the patient's family history of ovarian and breast cancer. The models included logistic regression and a three-layer back propagation neural network.

The prediction models were designed using 75% of the available data (156 cases), and were then tested using the remaining 25% of the cases (52 cases). The two models performed equally well for a test with one output variable (carrier versus non-carrier). The logistic regression model correctly predicted the mutation status for 40 of the 52 patients in the test group, including 63.2% of the carriers and 84.8% of the non-carriers. The neural network model correctly predicted the status of 41 of the 52 carriers in the test group, including 78.9% of the mutation carriers and 78.8% of the non-carriers.

In a second approach, we attempted to predict the mutation status at *BRCA1* and *BRCA2* concurrently, using polytomous logistic regression and using a three-layer neural network model with two outputs in its outer layer. The neural network performed better than the polytomous logistic regression in predicting mutation status at both genes. The polytomous logistic regression predicted 28.6% of the *BRCA1* carriers, 0% of the *BRCA2* carriers and 84.3% of the non-carriers. The two outputs neural network model predicted 71.4% of *BRCA1* carriers, 40.0% of *BRCA2* carriers, and 66.7% of non-carriers.

Familial Cancer Database: an example of low-cost world-wide electronic distribution of genetics software. *R.H. Sijmons¹, G.T.N. Burger², W. Weber³.* 1) Medical Genetics, Univ Groningen, Groningen, Netherlands; 2) Dept. of Pathology, Sazinson Foundation, Hoogetveen, Netherlands; 3) Chair UICC Familial Cancer and Prevention Project, Basel, Switzerland.

The Familial Cancer Database (FaCD) is free software to support differential diagnostics in clinical cancer genetics. The program has been released as part of the Familial Cancer and Prevention Project of the UICC (International Union against Cancer; <http://www.uicc.org/programmes/epid/familial.shtml>). Out of necessity to keep the distribution costs of FaCD low, the software is distributed by our organisation exclusively through the Internet, although individual users are free to distribute the software non-commercially to other health care professionals on CD-ROM's or diskettes. We have tried to draw attention to the FaCD web site (<http://facd.uicc.org>), which opened February 1st 2000, by submitting announcements to genetics and oncology journals as well as to medical web sites, by (poster) presentations and a journal article. However, we questioned whether such an approach and distribution system is indeed suitable for a project which aims at reaching potential users globally. We have therefore analyzed user-registration information, which includes country of residence and field of medical expertise. Results from such an analysis might also be useful for similar projects which aim at low cost distribution of genetics software (or information in general) to a global professional community.

By June 6th (5 months from the start), registrations for the software had been received from a total of 61 countries, including all Western-European and North-American countries as well as a wide range of countries from South-America, Eastern-Europe, Asia and Africa. Details on that range and on the fields of expertise of the users will be presented. We conclude that low cost distribution of oncogenetics software through the Internet may indeed reach potential users world-wide.

Cloning of a replicative senescence gene for immortal tumor cells. *G.P. Kaur, D.E. Reddy, R.S. Athwal.* Fels Cancer Research Inst, Temple Univ Sch Medicine, Philadelphia, PA.

Escape from senescence has been postulated to be a prerequisite for progressive tumor growth. Cells cultured from many tumors can proliferate indefinitely, while normal diploid cells become senescent after defined number of generations. Cellular senescence is expressed as a dominant phenotype over immortal cell growth. We have applied a functional-positional approach to map and isolate human genes that induce senescence in immortal tumor cell lines. A cell senescence gene, SEN16, identified on chromosome 16 by microcell mediated chromosome transfer into immortal mammary tumor cells, has been mapped within a genetic interval of 3-7cM at 16q24.3 (Reddy et al.1999, Oncogene 18:5100-5107). Six yeast artificial clones corresponding to candidate locus were identified and tested for functional complementation of immortal phenotype by introducing into mammary tumor cells. These studies led to the identification of a 360 kb YAC (Reddy et al. 2000, Oncogene 19:217-222) In order to construct a high-resolution map of the candidate region carrying the senescence gene, a human BAC library was screened with DNA markers to obtain relevant BAC clones, which were assembled in a contig based upon PCR analysis. Some selected BAC clones were retrofitted with a selectable marker and introduced into human and rat mammary tumor cells for functional complementation. These results identified a 85 kb BAC clone that carries the senescence gene. In following experiments, we isolated expressed sequences from the complementing BAC clone by exon trapping. Twelve exons were identified and their sequence comparison with databases has identified few partial cDNA clones. Experiments are in progress to determine the expression of these clones in tumor cell lines. In conclusion, we have mapped a senescence gene to a 85kb genomic clone and identified expressed sequences from this clone.

Construction of a BAC contig across the *NRC-1* region of chromosomal band 3p12. *J.M. McDonald¹, S.T. Lott¹, M. Lovell¹, S. Naylor², A.M. Killary¹.* 1) Experimental Lab. Medicine, Unit Texas MD Anderson CA Ctr, Houston, TX; 2) Dept. of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio.

Renal cell carcinoma (RCC) is the most common adult kidney neoplasm, and has a 5-year survival rate of less than 50%. Aberrations of chromosome 3 are found in more than 80% of RCC patients, and there is evidence for four different regions of 3p that could contain tumor suppressor genes. Evidence for one of these regions includes functional studies involving the introduction of 3p centric fragments into a RCC line SN12C.19. Subcutaneous injection of SN12C.19 into athymic nude mice resulted in the formation of tumors. Similiar injections of microcell hybrids that retained the subchromosomal region of 3p14-q11 caused suppression of tumor growth, based on the comparison of average tumor volume of the microcell hybrids (350-600mm³) to that observed from injection of the SN12C.19 parent cell line (900mm³). In contrast, other microcell hybrids that retained 3p12-q24 failed to suppress tumor growth. These experiments identified the region 3p14-p12 to contain the tumor suppressor locus nonpapillary renal carcinoma (*NRC-1*). Microsatellite analysis of the microcell hybrids and refinement of the physical map for chromosome 3 placed *NRC-1* within chromosomal band 3p12.

One of the major objectives of this project is to identify candidate loci for *NRC-1*. Sequencing of 3p12 is underway as part of the Human Genome Project, and BAC contigs for 3p12 are available. In order to construct a BAC contig that spans the region of *NRC-1*, a primary hybridization screen of the RPCI-11 BAC clone library was performed by using 12 markers from the *NRC-1* region. BAC contigs generated by this approach in combination with BAC contigs available through Washington University's fingerprint database will establish a minimal tiling path contig across the *NRC-1* critical region. BACs will be mapped by FISH onto the suppressed and unsuppressed hybrid clones as well as transfected into SN12C.19 using a functional genomic approach to identify candidates for *NRC-1*.

Screening of 20q13 Candidate Genes in Familial Prostate Cancer. *L. Wang¹, R.S. Berry¹, J.M. Cunningham¹, D.J. Schaid², S.N. Thibodeau¹.* 1) Lab Medicine & Pathology, Mayo Clinic, Rochester, MN; 2) Health Sciences Research, Mayo Clinic, Rochester, MN.

Prostate cancer (PC) is one of the most human cancers, occurring in as many as 15% of men in the United States. Recent studies suggest that hereditary PC is a complex disease, involving multiple susceptibility genes and variable phenotypic expression. Previously, four presumed PC susceptibility loci (HPC1, PCAP, CAPB, HPCX) have been identified through linkage studies. While conducting a genome-wide search on 162 North American families with ³ 3 members affected with PC, we found evidence for linkage to chromosome 20q13 with the largest 2-point LOD score of 2.8 for marker D20S196. The maximum multiple NPL score was 3.02 at D20S887. In an effort to clone PC susceptibility genes in this region, we constructed a physical and transcript map around this region covering 25.8cM from D20S119 to D20S149 with D20S196 in the middle. This map includes more than 104 BAC/PAC clones, 47 known genes, 17 genes for hypothetical proteins and 156 unigenes or ESTs. Based on their function and location on this map, we have selectively screened 13 known genes using conformation-sensitive gel electrophoresis (CSGE) in 60 patients from 30 families with the highest NPL score. These genes include STK15, UBE2V1, PD1, SNAI1, KIAA0784, KIAA0978, MOCS3, KIAA0939, DPM1, AIB1, KCNB1 and EYA2. No truncation type mutation (nonsense, frameshift, splice) was identified. However, numerous single nucleotide polymorphisms were identified in coding region of these genes. Specifically, the frequency of two polymorphisms in the STK15 gene tend to be different among familial PC, sporadic PC and normal controls. Currently, we are analyzing additional PC families to determine whether these polymorphisms contribute to familial prostate cancer. Additionally, we are continuing to screen other candidate genes in this region.

A prospective study on risk factors for hereditary breast cancer in a hospital-based series of breast cancer

patients. *M.G.E.M. Ausems¹, R.B. van der Luijt¹, V.C.M Koot², C.J.M. van der Sijs- Bos¹, H.F.A. Vasen³, R.P. van Helvoirt⁴, C.C. Warlam-Rodenhuis⁴.* 1) Dept Medical Genetics, Univ Medical Ctr Utrecht, Utrecht, Netherlands; 2) Comprehensive Cancer Ctr Utrecht, Netherlands; 3) Dept of Internal Medicine, Univ Medical Ctr Utrecht, Utrecht, Netherlands; 4) Dept of Radiotherapy, Univ Medical Ctr Utrecht, Utrecht, Netherlands.

To determine the prevalence of presumed risk factors for hereditary breast cancer, we performed a prospective study in 1000 breast cancer patients, and offered genetic counselling and mutation analysis of the BRCA1 and BRCA2 genes to a sub-group of patients. All patients had primary invasive breast cancer and received adjuvant radiotherapy between 1997 and 1999. For patient selection a check list with 8 risk factors was used. If patients had one or more risk factors, a pedigree was composed. Patients fulfilling the criteria for referral to the Family Cancer Clinic (FCC), were offered genetic counselling and DNA analysis. Selected regions of the BRCA1 and BRCA2 gene were analyzed. Risk factors for hereditary breast cancer were present in 515 of 1,000 patients. Less than half of the patients at risk for hereditary breast cancer were interested in genetic counselling. In total, 188 of 237 selected patients agreed with referral to the FCC. 164 patients (87%) came from families with breast cancer only; 18 (10%) from families with breast and ovarian cancer and 6 (3%) came from families with breast and ovarian cancer in one patient. Mutation analysis will be performed in 124 patients. To date, 6 BRCA1 mutations and 1 BRCA2 mutation have been identified in 105 tested patients. The frequency of mutations is low (7%) compared with breast cancer patients referred outside this study. The potential benefit of clinical genetics is not fully utilized as most of the patients would not have been referred outside this study.

The Needs of Male *BRCA1* and *BRCA2* Mutation Carriers. *D.E. Hanna¹, A. Liede¹, K. Metcalfe¹, E. Hoodfar¹, C. Snyder², C. Durham², H. Lynch², S.A. Narod¹.* 1) Women's College Hospital, Toronto, Canada; 2) Department of Preventive Medicine and Public Health, Creighton University, 2500 California Plaza, Omaha, NE.

The concerns of men at risk of inheriting a *BRCA1* or *BRCA2* mutation have received little attention. It was assumed that few men would be interested in predictive testing. However, men are likely to be affected emotionally by their female relatives diagnoses and they may harbor fears of developing cancer. Male *BRCA* carriers are at significantly increased risk of developing several cancers, including prostate, pancreatic and breast cancer. In addition, *BRCA2* carriers are at increased risk of gall bladder, bile duct cancer and cutaneous malignant melanoma. We have surveyed 57 of 67 eligible male *BRCA1* or *BRCA2* carriers from the University of Toronto and Creighton University. The men were asked about their motivations for seeking testing, involvement in family discussions of breast and ovarian cancer, change in cancer screening practices and overall satisfaction with the genetic counseling process. A range of emotions were reported in response to receiving a positive test result, including concerns for self (4%) and children/daughters (19%), feelings of sadness and fear (14%) and acceptance and relief (38%). Over half the subjects (53%) stated that the primary motivation for seeking genetic testing was for their children or family. The majority (88%) were included in past family discussions of breast and ovarian cancer risk but a smaller proportion (46%) was involved in family conversations about prophylactic surgery. Twelve percent stated that their family relationships were strengthened by this information, with an increased appreciation for their female relatives' experience. Almost one-third of respondents indicated that they would be interested in taking part in an information/support group. Over half of the men (56%) indicated that they thought about their risk of developing cancer (29% of these regularly or daily). Half of the men stated that their cancer screening practices had changed as a result of learning their carrier status, two-thirds having regular PSA for prostate cancer surveillance. Overall, high levels of satisfaction with genetic counseling were reported.

Over-representation of a germline variant in *GFRA1*, encoding a RET co-receptor, but not *GFRA2* and *GFRA3* in patients with sporadic medullary thyroid carcinoma. *O. Gimm*¹, *H. Dziema*¹, *J. Brown*¹, *C. Hoang-Vu*², *R.*

*Hinze*³, *L. Mulligan*⁴, *H. Dralle*², *C. Eng*¹. 1) Human Cancer Genetics Program, The Ohio State University, Columbus, OH, USA; 2) Dept. of General Surgery, Martin-Luther-University, Halle, Germany; 3) Institute of Pathology, Martin-Luther-University, Halle, Germany; 4) Dept. of Pathology and Paediatrics, Queens University, Kingston, ON, Canada.

In contrast to hereditary MTC, little is known about the etiology of sporadic MTC. Somatic gain-of-function mutations of RET are found in an average of 40%. Mutation analysis of GDNF, encoding one of the ligands of RET, has not revealed any mutations. We analyzed 31 sporadic MTC for somatic and germline variants in *GFRA1*, 2, and 3, encoding RET co-receptors. Although there were no somatic mutations in all three genes, a rare polymorphic sequence variant (193C>G) in the 5-UTR of *GFRA1* was found in 15% of MTC alleles. Three patients were heterozygous (het), another 3 homozygous (hom) for the G variant. We did not find this variant in any of 62 alleles from a region-matched control group ($p < 0.05$). Since this variant lies within a CpG-rich region, we analyzed this region for methylation. We found various degrees of methylation in germline and tumor DNA. Interestingly, methylation in tumor DNA was higher than in the corresponding peripheral leucocyte DNA ($p < 0.01$). No association between methylation status and expression of *GFRA1* at the transcript level could be identified. However, tumors harboring the rare G variant seemed to have higher expression of *GFRA1* transcript ($p = 0.065$). Immunostaining for GFRA1 was suggestive of overall stronger cytoplasmic staining intensity in het patients and stronger nuclear staining intensity in hom patients as compared to tumors from patients without the rare G variant. In conclusion, mutation analysis of *GFRA1*, 2, and 3 revealed over-representation of a rare germline sequence variant in *GFRA1*, which showed a trend towards association with differential expression levels and patterns at both transcript and protein levels. Whether this or other mechanisms contribute towards low penetrance susceptibility remains to be fully worked out.

Somatic and occult germline mutations in *SDHD*, a mitochondrial complex II gene, in apparently sporadic pheochromocytoma. *M. Armanios*¹, *O. Gimm*¹, *H. Dziema*¹, *H.P.H. Neumann*², *C. Eng*¹. 1) Human Cancer Genetics Program, Ohio State Univ, Columbus, OH; 2) Dept. of Medicine, Albert-Ludwigs-University, Freiburg, Germany.

Most pheochromocytomas, also known as adrenal paragangliomas, are sporadic but about 10% are associated with inherited syndromes. While the etiology of most familial pheochromocytomas is well known, little is known about the genetic basis of the more common sporadic type. Recently, germline mutations of *SDHD* were found in patients with hereditary paragangliomas, most often found in the carotid body. We therefore sought to determine the role of *SDHD* in the development of sporadic pheochromocytomas. We analyzed the coding sequence of *SDHD* as well as flanking markers (D11S1347, D11S1986) for LOH. Among 18 samples, we identified four heterozygous sequence variants (3 germline, 1 somatic). One germline mutation, IVS1+2T>G (absent among 78 control alleles), is predicted to cause aberrant splicing and is likely of functional significance. In addition, this patient had recently been diagnosed with a tumor of the carotid body, a probable paraganglioma. The second germline mutation (c.112C>T) was found in a patient with extra-adrenal pheochromocytoma, resulting in a nonsense alteration (R38X) which predicts a likely dysfunctional peptide. Another patient with malignant, extra-adrenal pheochromocytoma was found to have germline missense mutation c.34G>A (G12S). However, this sequence variant was also found in 1 of 78 control alleles. The only somatic mutation, c.242C>T (P81L), was found resulting in a change of a highly conserved proline residue among four eukaryotic multicellular organisms. This mutation is identical to germline mutations described in two families with hereditary paraganglioma. Overall, LOH in at least one of the two markers was found in 13 tumors (72%). All tumors already harboring intragenic *SDHD* mutations also showed LOH. Our results indicate that *SDHD* plays a role in the pathogenesis of pheochromocytoma. Given the 17% germline mutation frequency in this set of apparently sporadic pheochromocytoma cases, our observations might suggest that all such patients should be routinely subjected to *SDHD* mutation analysis.

Knockout of the Mouse Men1 Gene Gives a Lethal Phenotype in the Heterozygous Chimera. *J.S. Crabtree¹, P.C. Scacheri¹, E.A. Novotny¹, L. Garrett-Beal¹, A. Chen¹, K.A. Edgemon², S.J. Marx², A.M. Spiegel², S.C. Chandrasekharappa¹, F.S. Collins¹.* 1) NHGRI/NIH, Bethesda, MD; 2) NIDDK/NIH, Bethesda MD.

Multiple Endocrine Neoplasia, type 1 (MEN1) is an autosomal dominant familial disorder characterized by tumors of the parathyroid, gastrointestinal endocrine tissues and the anterior pituitary. Germline, heterozygous loss-of-function mutation followed by somatic loss of the normal allele leads to tumor formation in subjects with MEN1. This suggests that the MEN1 gene is a tumor suppressor, but the specific function of the nuclear protein, menin, encoded by the gene is unknown. To elucidate the function of menin, a conventional mouse knockout model was developed. To our surprise, the Men1 conventional knock out was lethal in heterozygous, chimeric animals. Removal of embryos revealed late gestational lethality, due to omphalocele, in what appeared to be a dose dependent manner based on the degree of chimerism. Southern analysis of embryo DNA indicated the homologous recombination was specific and did not affect the primary structure of neighboring genes. Northern analysis of conventionally targeted embryonic stem cell RNA revealed the presence of an abundant, novel transcript of 2.1kb. Wildtype transcripts are 2.7kb and 3.1kb, and the small size of this aberrant transcript suggests bidirectional activity from the PGK promoter (used to drive neomycin for ES cell selection) in the antisense orientation. RT-PCR data supports this postulated bidirectional activity of the PGK promoter. While as yet we have not been able to detect an aberrant protein on Western blots from targeted ES cells, we hypothesize that the PGK-driven transcript is producing a dominant negative effect through the production of an amino-terminal truncated protein product.

Identification of Human Papillomavirus DNA sequences in paraffin-embedded Retinoblastoma tissue. H.

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Retinoblastoma is the most frequent intraocular tumor in children and the main cause of enucleation. The etiology is diverse and includes mitotic nondisjunction and duplication of the mutated gene or loss of the wild-type chromosome, mitotic recombination between the RB1 locus and the centromere or punctual mutations. Although there are no antecedents of Human Papillomavirus (HPV) infection and Retinoblastoma, there are data about the transmission of HPV DNA from the sperm cells to the embryo, which can possibly dysregulate the retinal cells, causing Retinoblastoma. The purpose of this study was to investigate the presence of the HPV DNA in paraffin-embedded Retinoblastoma tissue. 56 samples in a period of time of 10 years (1987-1997) were obtained. The analysis by means of Polymerase Chain Reaction with consensus primers showed 44 positive samples (78.5%) and 12 negative (21.5%). We also tested specific amplification of HPV 16 and 18 in the positive samples, resulting negative for both. Our results suggest that the HPV could be involved in some cases of Retinoblastoma. Further studies remain in order to establish the types of HPV involved in this tissue sample preliminary survey (possibly low or medium-risk HPVs, because of the absence of High-risk HPV) as well as to define the role of HPV in the etiology of Retinoblastoma.

An interaction of alcohol intake and family history on breast cancer risk? *C.M. Vachon, J.R. Cerhan, R.A. Vierkant, T.M. Therneau, T.A. Sellers.* Health Sciences Research and Mayo Clinic Cancer Center, Mayo Foundation, Rochester, MN.

There is significant heterogeneity in studies of alcohol consumption and breast cancer. One explanation for the variability in results could be the presence of effect modifiers, such as a family history of cancer. We examined the association of alcohol and breast cancer risk within a cohort of 426 multigenerational families who were ascertained through a breast cancer proband at the University of Minnesota Hospital and Clinic between 1944 and 1952. We evaluated whether alcohol use among sisters, daughters, nieces and granddaughters of breast cancer probands was a stronger risk factor than among women who married into these families. Lifetime frequency of usual alcohol intake (daily, weekly, < weekly, never drinker), breast cancer risk factors, and medical history information were collected through a telephone interview between 1991 and 1995 with either a living individual or a surrogate. Relative risks (RR) and 95% confidence intervals were estimated through Cox proportional hazards models using each woman's age as her own timescale for the baseline risk. Analyses were adjusted for smoking status, birth cohort and type of information (surrogate or self-report). A total of 9032 blood relatives and marry-ins with 558 cases of breast cancer were available for analysis. In the entire 426 families, there was a suggestion of an interaction of relationship to the proband and frequency of alcohol consumption on breast cancer risk ($p_{\text{intx}}=0.14$). Among first- and second-degree relatives of the proband, daily drinkers had an increased risk of breast cancer compared to never drinkers (RR for daily vs. never drinkers among 1st degree relatives=2.45(1.20,5.02); RR for 2nd degree=1.27(0.73,2.20)), but this increase was not evident in marry-ins reporting daily use of alcohol (RR=0.90 (0.42,1.90)). The findings based on the subset of 132 high-risk families with 3 or more breast and/or ovarian cancers were similar to analyses based on all 426 families ($p_{\text{intx}}=0.07$). Thus, alcohol associated risks of breast cancer may be increased among women with a family history.

Evidence for a morphological continuum in inherited breast cancer: Implications for genetic testing. *M.J.*

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In testing for hereditary breast cancer susceptibility, only women with invasive breast or ovarian cancer are presumed to be gene carriers. Currently, neither in-situ breast cancer nor atypical hyperplasias are considered as clinical evidence of breast-ovarian syndrome, nor are these diagnoses used to predict carrier status within at-risk families. This reflects lack of evidence that breast cancer develops along a recognized morphological continuum from precursor lesions. We analyzed malignant breast cancer tissue and potentially preneoplastic lesions (PPL) from three individuals bearing BRCA1 mutations for p53 mutations. We used immunohistochemistry for p53 protein, followed by mutational analysis of exons 2-11 of the p53 gene using the Affymetrix p53 gene chip assay. In addition to normal and malignant breast tissue from the three patients, the PPL included hyperplasia and papilloma in a 2178 C->T BRCA1 mutant (case 1); papilloma in a 3519 G->T BRCA1 mutant (case 2); and hyperplasia and apocrine metaplasia in a 4154 delA BRCA1 mutant (case 3). Breast tumors from all three cases had unique disease-associated exon 6 p53 mutations. In case 1, the p53 mutation observed in the tumor was also present in the papilloma but not in the hyperplasia; in case 2, the papilloma and the tumor had the same mutation; and in case 3, areas of apocrine metaplasia from two separate tissue blocks (one adjacent to tumor, and one without tumor) showed the same p53 mutation as that in the tumor. These results indicate that p53 mutations occur early in the evolution from proliferative breast lesions to malignancy, and that clonal molecular alterations provide evidence for an evolutionary continuum of those tissue changes in BRCA1 mutation-bearing women. We suggest that in high-risk families without a previously identified BRCA1 mutation and lacking available affected family members with breast or ovarian cancer, women with proliferative breast lesions may qualify as candidates for carrier testing.*BRCA1*.

Risk of other cancers among French Canadians family members of BRCA1 and BRCA2 gene carriers. P.

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Several studies have suggested an increased risk of some cancer sites, other than breast and ovarian among the blood relatives of BRCA1 and BRCA2 gene carriers. We have carried out a preliminary analysis of 41 mutation positive French Canadian families (including 1001 first degree relatives and 220 probands) in Quebec, Canada and found an excess of cancers other than breast and ovarian such as: [any cancer: SMR=1.23; 95%CI (1.01-1.49); P=0.046 for male and SMR=1.74; 95%CI (1.32-2.25); P=0.00013 for female] prostate cancer with a SMR of 1.61; 95%CI (1.01-2.45); P=0.045; colorectal cancer SMR = 1.92; 95%CI (1.14-3.04)., P=0.016 in males and SMR=2.58; 95%CI (1.50-4.12), P=0.011 in females. The SMR for thyroid cancer in male relatives was 9.98 with a 95%CI of (2.72-25.57), P=0.002. We also observed significantly higher risk of cervical cancer among female relatives of gene carriers, SMR=3.43, 95%CI (1.11-8.01), P=0.03. Lymphoma-Hodgkins disease was also higher among gene female relatives of gene carriers, indicating a SMR=5.35; 95%CI (1.46-13.70), P=0.016. The preliminary results of our study suggests, higher frequency of certain types of cancer (other than breast and ovarian) among BRCA1 and BRCA2 gene carriers.

Linkage analysis of 173 prostate cancer families using markers that span the region of 20q containing the novel prostate cancer susceptibility locus HPC20. *C.H. Bock¹, J.M. Cunningham², S.K. McDonnell², E.M. Lange¹, D.J. Schaid², B.J. Peterson², K. Brierley¹, R. Pavlic¹, J. Schroeder², J. Klein², A. French², A. Marks², S.N. Thibodeau², K.A. Cooney¹.* 1) Departments of Epidemiology, Biostatistics, Internal Medicine and Surgery, University of Michigan, Ann Arbor, MI; 2) Departments of Laboratory Medicine and Pathology and Health Sciences Research, Mayo Clinic/Foundation, Rochester, MN.

Four prostate cancer predisposition genes have been identified through family-based linkage approaches: HPC1, PCAP, CAPB and HPCX. A recent linkage study of 162 Caucasian prostate cancer families described evidence for a previously unreported prostate cancer predisposition gene termed HPC20 on 20q13. To confirm the potential contribution of this locus to prostate cancer susceptibility, we studied 173 unrelated prostate cancer families participating in the University of Michigan Prostate Cancer Genetics Project. These families have at least three prostate cancer diagnosed members or two prostate cancer diagnosed members under age 55 years. 582 DNA samples were genotyped using a panel of 17 polymorphic markers spanning 98.5 cM and containing the HPC20 candidate region. Data were analyzed using both model-free (NPL) and parametric linkage methods with GENEHUNTER. Analysis of all 173 families resulted in a maximal NPL Z-score of 0.63 with a corresponding one-sided p-value of 0.26 at marker D20S171. Stratification analysis revealed increased evidence for linkage in the subset of 18 families without male-to-male transmission, <5 affected members, and age at onset > 66 years (NPL Z-score of 1.27, p-value 0.11 at marker D20S889). In the original report by Berry et al. (Am J Hum Genet. vol. 67, p.82-91, 2000) the strongest evidence for linkage occurred in an identically defined subset. Highest evidence for linkage in our study was seen in the subset of 17 African-American families (NPL Z-score of 1.95, p-value 0.03, at marker D20S893). Overall, our linkage study provides support for the existence of a prostate cancer susceptibility gene at 20q13, although genetic complexity will likely require further confirmation, perhaps by a meta-analysis.

Genome-wide scan of high-risk prostate cancer families with breast cancer reveals novel prostate-breast loci. *J.L. Stanford*¹, *M.D. Badzioch*², *S. Kolb*¹, *M. Peters*¹, *M. Janer*³, *E.L. Goode*², *M. Gibbs*¹, *L. Hood*³, *E.A. Ostrander*¹, *G.P. Jarvik*². 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) University of Washington, Seattle, WA; 3) Institute for Systems Biology, Seattle, WA.

Hereditary prostate cancer (PC) is a complex disease that may be associated with other cancers in some families. Epidemiological and genetic studies have reported familial clustering of prostate and breast cancers. As part of a genome search for prostate cancer genes, we performed linkage analyses in 27 families with 3 or more affected men with PC who had at least 1 first-degree relative with breast cancer (BC). To maximize homogeneity, these families were stratified by the pattern of BC (1 case, n=14 families; ³ 2 cases, n=6; any ovarian cancer, n=7). Families were stratified into early (<66 yrs.) or later (≥66 yrs.) onset PC based on median age at diagnosis. GENEHUNTER was used to compute multipoint NPL scores for PC linkage using 380 genomic scan markers. Stratified analyses revealed 3 chromosomal regions with NPL scores ³ 3.0: 1) chr. 7q, NPL=3.98 (p=0.002) for markers D7S1826 through D7S1805 in later-onset PC families with BC and ovarian cancer; 2) chr. 12q, NPL=3.00 (p=0.010) at marker D12S392 in all families with more than one relative with BC; and 3) chr. 14q, NPL=3.09 (p=0.006) at marker D14S306 in early-onset PC families with one first-degree relative with BC. None of these groups had significant evidence of linkage of PC to chr. 13 or chr. 17. These data provide evidence for loci on chromosomes 7q, 12q, and 14q that may be involved in both prostate cancer and breast cancer susceptibility.

Genes within the common fragile sites are down-regulated in ovarian cancer. *M.J Ferber¹, S.R. Denison², N.A. Becker², J. Lee³, J. Lillie³, L.C. Hartmann⁴, V. Shridhar², D.I Smith².* 1) Biochemistry/Molecular Biology, Mayo Foundation, Rochester, MN; 2) Division of Experimental Pathology, Mayo Foundation, Rochester, MN; 3) Millenium Predictive Medicine, Cambridge, MA; 4) Division of Oncology, Mayo Foundation, Rochester, MN.

We have been using transcriptional profiling to analyze gene expression in primary ovarian tumors. Using a 25,000 gene microarray filter from Millenium Predictive Medicine, we analyzed 15 primary ovarian tumors to identify genes that were aberrantly regulated relative to uncultured ovarian epithelial cells. Many genes were found to be consistently aberrantly regulated in the ovarian tumors. An analysis of genes that were down-regulated at least 5 fold in most of the ovarian tumors profiled revealed that many of these genes were derived from chromosomal bands known to contain common fragile sites. We chose ten genes down-regulated in the ovarian tumors that were derived from chromosomal bands known to contain common fragile sites and first confirmed their expression using RT-PCR and Northern Blot analysis. To determine if the verified genes were actually derived from within a common fragile site, we isolated bacterial artificial chromosomes (BACs) spanning these genes and used these as probes for FISH analysis with aphidicolin-induced metaphase chromosomes. BAC clones spanning 6 of the 10 genes analyzed were found to span previously uncloned common fragile sites. We have fully characterized three of these new common fragile sites and determined their size and the position of the down-regulated gene relative to aphidicolin-induced breakage in these regions. We are currently analyzing an additional 10 genes to determine if they also reside on common fragile sites. This has therefore proven to be a powerful technique to quickly localize previously uncloned common fragile sites. A more important question is whether or not many of the genes residing within common fragile sites are inactivated during the development of ovarian, and possibly other, cancers.

High-throughput gene copy number analysis in 4700 tumors: FISH analysis on tissue microarrays identifies multiple tumor types with amplification of the MB-17A gene, a novel amplified gene originally found in breast cancer. C.L. Andersen^{1, 2}, O.M. Monni¹, J. Kononen¹, M. Barlund³, C. Bucher⁴, P. Hass⁴, A. Nocicito⁴, H. Bissig⁴, G. Sauter⁴, O-P. Kallioniemi¹, A. Kallioniemi¹. 1) Cancer genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Danish Cancer Society, Århus, Denmark; 3) Institute of Medical Technology, University of Tampere and Tampere University Hospital, Finland; 4) Institute of Pathology, University of Basel, Switzerland.

We recently developed a novel technique for high density arraying of hundreds of 0.6 mm diameter tissue cylinders (Kononen et al, Nat Med 4: 844, 1998). Consecutive sections cut from such a tissue microarray (TMA) on a microscope slide provide a template for a high-throughput analysis of multiple DNA, RNA and protein targets in each of the hundreds of tissue specimens on the array. Here we constructed a set of TMAs for high throughput analysis of interesting genes in specimens from many different tumor types. Four thousand seven hundred tumor specimens, representing 135 different tumor types were arrayed into ten TMA blocks. Sections from these blocks provide a template for a systematic screening of most malignant tumor types. The MB-17A gene was recently identified by cDNA microarray experiments to be amplified, overexpressed and rearranged in breast cancer (see ASHG abstracts Monni et al. and Kallioniemi et al. 2000). In order to study the importance of this gene in cancer development, across all tumor types, we performed FISH with a MB-17A gene probe on the multi-tumor TMA. As expected, we found high-level amplification of MB-17A most often in breast cancer, with a frequency of 13/129 (10%). Other tumor types with MB-17A amplification included cancers of the endometrium, ovary, thyroid, parathyroid, urinary bladder and squamous cell carcinoma of the skin and melanoma. In summary, our results indicate that MB-17A maybe involved in the development of multiple different tumor types. High-throughput tissue microarray technology allows screening of thousands of tumors at a time and thereby facilitates rapid translation of gene discoveries to clinical and diagnostic applications.

A search for germline APC mutations in early onset colorectal cancer or familial colorectal cancer with normal DNA mismatch repair APC. *L.A. Boardman¹, S. Schmidt³, N.M. Lindor², L.J. Burgart³, J.M. Cunningham³, T.L. Price-Troska³, K. Snow³, D.A. Ahlquist¹, S.N. Thibodeau³.* 1) Department of Internal Medicine, Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN; 2) Department of Medical Genetics, Mayo Clinic, Rochester, MN 55905; 3) Department of Laboratory Medicine and Pathology, Mayo Clinic Rochester, MN 55905.

Twenty percent of colorectal cancers (CRC) arise in people who have a family history of CRC in at least one other relative. Though a fraction of these are explained by two well-described autosomal dominant syndromes-5% by hereditary nonpolyposis colorectal cancer (HNPCC) and 1% by familial adenomatous polyposis (FAP), the cause of the remaining 14% of familial aggregates of CRC is unknown. Many cases of HNPCC are due to germline mutations in DNA mismatch repair genes, leading to the tumor phenotype of microsatellite instability (MSI), and most cases of FAP are caused by germline APC mutations. To date, non-FAP familial CRC aggregates have not been evaluated for germline APC mutations. In this study, we examined the involvement of germline APC mutations in 79 individuals with CRC who had either early age onset of their cancer (age <50 years) and/or a family history of CRC. Cases with FAP or HNPCC due to defective mismatch repair were excluded from the study. Using conformation sensitive gel electrophoresis (CSGE) and the protein truncation test (PTT) as the screening methods, functionally significant germline mutations were not detected for any of the cases. An apparently silent polymorphism resulting in a one base pair alteration of A>G (Proline> Proline) in exon 4 was observed. Additionally, four intervening sequence (IVS) alterations were detected: 1) IVS2 (-53) t>c in 3 cases; 2) IVS4 in 3 cases (-17) ins T; 3) IVS5 (+32) t>c in 16 cases and 4) and IVS 5(+33) g>a in 1 case. All appeared to be polymorphisms present in similar proportions in an average risk population. We conclude that germline APC mutations do not account for familial MSS CRC associated with few synchronous polyps.

Imbalance in wild-type hMSH2:hMLH1 protein ratio in lymphocytes identifies colorectal cancer (CRC) patients with hereditary nonpolyposis colon cancer (HNPCC) traits. Z.P. Gao¹, Z.Q. Gao¹, S. Burkholder¹, T. Zhang¹, J.Z. Fields², A. Kairo¹, S. Ehrlich¹, B.M. Boman¹. 1) Medical Genetics, thomas jefferson university, philadelphia, PA; 2) CA*TX Inc., Gladwyne, Philadelphia, PA 19035.

Identifying carriers of HNPCC traits can save lives and health care dollars. However, current genetics tests for HNPCC is costly and time consuming. Therefore, the need is to develop a more feasible assay to detect a germline HNPCC-causing mutation, particularly one that is sensitive, rapid, easy & inexpensive. Our approach to the problem of detecting HNPCC was to develop a quantitative immunoassay for wild-type DNA mismatch repair (MMR) protein levels (hMSH2 and hMLH1). Mutations in hMSH2 and hMLH1 account for the vast majority (>90%) of detectable germline mutations in HNPCC kindreds. And most (>70%) germline hMSH2 and hMLH1 mutations lead to a truncated protein product. We hypothesized that cells carrying a germline, truncation-causing, hMSH2 or hMLH1 mutation will have a 50% reduction in the corresponding full-length protein product. To test proof of principle for our assay, we used western blot analysis to estimate hMSH2 and hMLH1 protein levels in lymphoblastoid cell (WBC) lines from CRC patients. We tested 42 WBC lines established from CRC patients in our Familial Colorectal Cancer Registry and 8 WBC lines from healthy, unaffected individuals. Western blots were done using antibodies against the carboxyl end of both hMSH2 and hMLH1 proteins. All of the samples from healthy unaffected individuals had, on western blots, a) clearly identifiable bands for hMSH2 and for hMLH1 and b) nearly identical hMSH2/hMLH1 ratios. In 7 of the 42 WBC lines from CRC patients, we found decreased expression of hMSH2 or hMLH1 on western blot analysis and all 7 of these, subsequently, showed evidence of a mutation in corresponding MMR gene. Moreover, where DNA was available from fresh lymphocytes (5 of the 7), we found evidence for the mutation. Together these data demonstrate i) that our assay can be used to identify individuals with the HNPCC trait and ii) that this can be done practically and inexpensively.

Identification and characterisation of the familial cylindromatosis gene. *G.R. Bignell¹, W. Warren², S. Seal², M. Takahashi², E. Rapley², R. Barfoot², P. Biggs², A. Ashworth², M. Stratton².* 1) Team 78, The Sanger Centre, Hinxton, Cambridgeshire, UK; 2) Cancer Genetics Section, Institute of Cancer Research, Sutton, Surrey, UK.

We report the cloning of the gene for Familial Cylindromatosis (*CYLD*). Familial Cylindromatosis is an autosomal dominant genetic predisposition to multiple tumours of the skin appendages. The susceptibility gene has previously been localised to chromosome 16q12-q13 and has the genetic attributes of a tumour suppressor gene/recessive oncogene. The critical evidence for involvement of the *CYLD* gene in familial cylindromatosis is the identification of 21 germline mutations. We have also identified six somatic mutations, five from familial cylindromas and one from a sporadic cylindroma. All of the germline and somatic mutation are predicted to cause early protein termination. The *CYLD* gene has also been screened through a bank of other tumours, however, no mutations were detected from this set. Analysis of the protein sequence reveals three regions with homology to Cytoskeletal Associated Protein-Glycine conserved (CAP-GLY) domains, found in proteins that co-ordinate the attachment of organelles to microtubules, and homology to the catalytic domain of Ubiquitin Carboxy-terminal Hydrolase type 2.

Functional evidence for multiple tumor suppressor genes on the short arm of chromosome 8. *G. Chenevix-Trench*¹, *J. Arnold*¹, *P. Wilson*¹, *D. Trott*², *A. Cuthbert*², *R. Neubold*². 1) Queensland Inst Medical Res, Brisbane, Australia; 2) Brunel University, Uxbridge, UK.

The short arm of chromosome 8 frequently undergoes loss of heterozygosity in many different solid tumors, and homozygous deletions have been reported in prostate and squamous cell carcinomas. We have previously provided functional evidence of a tumor suppressor gene on this chromosome by the transfer of chromosome 8 into colorectal cancer cell lines by microcell fusion. Spontaneous deletions of the donor chromosome allowed us to map the location of the putative tumor suppressor region to a 5.2 Mb region at 8p22-23. We have now transferred chromosome 8 into ovarian (HEY) and breast (21MT and T47-D) cancer cell lines. HEY/8 hybrids containing all of the donor chromosome 8 grew normally in vitro but showed significantly reduced tumor formation in athymic mice compared to those that only contained the long arm of the donor chromosome. Analysis of hybrids containing only part of the donated 8p have allowed us to map two regions of suppression at 8p12-21 and 8p23. We have also obtained 17 21MT/8 and 5 T47-D/8 hybrids. All of these hybrids share in common the exclusion of several regions on the short arm of the donor chromosome. This suggests that they might contain tumor suppressor genes that convey a strong selective disadvantage in vitro. These data suggest that there are several tumor suppressor genes on the short arm of chromosome 8 and efforts are underway to narrow down their location prior to gene identification.

Towards identification of a senescence-associated gene / tumor suppressor gene (TSG) in the NRC-2 locus in human chromosome band 3p14. *B. Opalka¹, W. Bardenheuer¹, G. Marquitan¹, N. Werner¹, K. Juelicher¹, H. Topal¹, I. Horikawa², J.C. Barrett², J. Schuette¹.* 1) Innere Klinik (Tumorforschung), Universitaet (GH) Essen, Essen, NW, Germany; 2) NIEHS, Research Triangle Park.

Chromosomal alterations in human chromosome region 3p14 have been found in numerous tumor entities including lung cancer (LC) and renal cell carcinoma (RCC). Results of functional investigations as well as data concerning structural aberrations suggest the presence of at least one TSG in 3p14. The FHIT gene in 3p14.2 and the WNT5A gene in 3p14.3-21 have been shown to reveal TSG function in vitro and/or in animal models while mutations of these genes have been rarely detected in LC or RCC. We have previously established functional complementation assays using a YAC contig covering chromosomal band 3p14 and neighboring regions. Following retrofitting of YACs for the introduction of a mammalian selectable marker we established the YAC transfer by spheroplast fusion into a human RCC line showing a cytogenetically detectable deletion within 3p13-23. Using this approach we identified a 530 kb YAC clone within 3p14.2 which induced cellular senescence in vitro and reveals sustained suppression of tumorigenicity of transduced RCC cells in nude mice. This activity which maps differently from the FHIT gene and the WNT5A gene defines a novel TSG locus, NRC-2, in 3p14. Genomic sequencing was performed using PAC clones. Including data from the human genome project 60-80% of the entire YAC sequence are available now. Primers were synthesized corresponding to 6 ESTs and 15 predicted exon sequences as well as for 8 exon sequences identified in exon trapper experiments and are currently used to screen different cDNA libraries. These efforts should allow the isolation of the gene(s) responsible for the NRC-2 locus activity. Supported by Deutsche Forschungsgemeinschaft and foundation VerUm.

Two deletions in regions 6q22-23 and 6q25-27 are associated with immortalization of SV40 transformed cells. *J. Liu, A.K. Sandhu, N. Rane, R.S. Athwal.* Department Of Pathology, Fels institute, Temple University School of Med. Philadelphia, PA.

We have applied a 'Functional-Potential' approach to identify cell senescence genes on human chromosomes. Microcell mediated transfer of a gpt tagged intact human chromosome 6 or a part of the long arm (6q14-qter) restored senescence in human (Sandhu et al. 1994, PNAS 91: 5498-5502) and mouse SV40 immortalized fibroblasts. Segregation of the transferred chromosome 6, with the loss of gpt tag, led to the resumption of indefinite cell proliferation. While microcell hybrids were maintained in the MX selection medium, for the retention of the donor chromosome, immortal revertant clones arose among senescent cells. Reversion to immortal growth could result from the loss of the expression of the senescence gene due to a mutation or a deletion. Analysis of the revertant clones for the loss of the DNA markers, mapped in the region 6q22-qter, identified two deletions in the region 6q22-27. The results of this study revealed two independent deletions located at 6q22-23 and 6q25-27, suggesting the location of two senescence genes on the long arm of chromosome 6. In order to further define the position of the senescence gene(s), we have identified YAC and BAC clones corresponding to the deleted markers, by human genome data base search. A high resolution physical map of the region containing the senescence gene has been developed. Candidate BAC clones will be tested for the restoration of senescence by introduction into SV40 immortalized human and rodent cells.

BRCA2 mutations in African Americans. *Y.M. Kanaan¹, E. Kpenu¹, K. Utley¹, L.C. Brody², G.M. Dunston¹, C. Whitfield-Broome¹.* 1) Howard University College of Medicine, Departments Microbiol., Biochem.& Mol. Biol.; Cancer Center, Humam Genome Center, Washington, DC.20059; 2) National Human Genome Res. Inst., Bethesda MD 20892-4442.

Since the identification of the BRCA2 breast-ovarian cancer susceptibility gene, mutation analyses have been carried out in various populations revealing ethnic-specific mutations. In order to identify BRCA2 mutations in African Americans, seventy-five breast cancer patients from families at high risk of hereditary breast cancer were studied. The entire coding regions and flanking introns of BRCA2 have been screened for germline mutations by single stranded conformational polymorphism or the protein truncation test, followed by DNA sequencing. Eight protein truncating, pathogenic mutations have been detected. Four (1991delATAA, 1993delAA, 2001delTTAT, 8643delAT) of the eight pathogenic mutations observed in African Americans have not been previously described. Therefore, half of the pathogenic mutations observed are unique to African Americans. The other four pathogenic mutations (1882delT, 2816insA, 4075delGT, 4088delA) detected in African Americans have been previously reported in Caucasians. Two of the pathogenic mutations, 1991delATAA and 2816insA, were detected in male breast cancer patients, consistent with previous studies showing an association of BRCA2 with male breast cancer. One-half of the pathogenic mutations were identified in women diagnosed with breast cancer under the age of 40 with or without a family history of the disease. One rare missense variant of unknown functional significance was detected. Numerous polymorphisms and non-coding variants were observed. Considering our work and that of others, many different pathogenic mutations and many variants of unknown significance are observed in African Americans; therefore, BRCA2 genetic testing in high risk African American families needs to involve the entire coding and flanking sequences of the gene. This work was supported by the U.S. Army Medical Research and Materiel Command under DAMD17-98-1-8106 and in part by the Komen Found.

Genome wide variation in LD : Insights for association studies. *P.E. Bonnen¹, D. Triikka¹, Z. Fang¹, M.M. Weil⁴, M. Kimmel², R. Chakraborty³, D.L. Nelson¹.* 1) Baylor Coll of Med, Houston, TX; 2) Rice Univ, Houston, TX; 3) Univ of Texas Health Science Ctr, Houston, TX; 4) MD Anderson Cancer Ctr, Houston, TX.

Polymorphisms in the human population may lead to functional variants of genes that lend increased or decreased risks for cancer. Our strategy for detecting such risk is to develop haplotypes for genomic regions (130-180kb) containing candidate cancer genes and to perform association studies in cancer populations. We used a resequencing approach to uncover SNPs (8-14) spanning the length of each locus of interest (ATM, BLM, WRN, and RECQL). We then genotyped an ethnically defined control population (~300) for these SNPs and using an EM algorithm predicted haplotypes and their frequencies for each locus. A striking difference between genes was immediately apparent with ATM having 22 haplotypes while BLM, WRN, and RECQL had 50, 56, and 47 respectively. Investigation with the four gamete test revealed reduced recombination at the ATM locus. The most extreme case of which was found in the ATM Caucasian population where there was no evidence for recombination in the entire 140 kb covered by these haplotypes. Examination of LD across these loci followed the same trend with ATM showing extensive LD. To investigate the utility of these haplotypes for association studies we ventured three model studies. First, we established the ability of our BLM haplotypes to detect a founder mutation (blmAsh) for Bloom Syndrome in the Ashkenazi Jewish population. Interrogation of nine Bloom Syndrome patients revealed the mutation occurred strictly on BLM haplotype 25. Next, we demonstrated the efficacy of the ATM haplotypes for detecting polymorphisms by simulating an association study with coding SNPs (cSNPs) in the ATM gene. We genotyped samples known to have one of three particular cSNPs as our "case" population (n= 84) and samples without the cSNPs as controls (~500). Each cSNP was detected by association with an ATM haplotype. A similar study was done with the WRN gene haplotypes and six cSNPs. No haplotype associations were found. Frequency of mutation and haplotype, strength of association as well as extent of LD influence the ability to ascertain risk through association studies.

Association of 5-alpha-reductase gene (SRD5A2) with prostate cancer. *N. Mononen¹, P.A. Koivisto¹, T. Ikonen¹, K. Syrjäkoski¹, J. Schleutker¹, E. Hyytinen¹, T.L.J. Tammela², O.-P. Kallioniemi¹.* 1) Dept Cancer Genetics, Inst Medical Technology, Tampere, Finland; 2) Division of Urology, Tampere University Hospital, Tampere, Finland.

The molecular mechanisms underlying prostate cancer development are incompletely understood. However, androgens are suspected to play a critical role. Human 5-alpha-reductase (SRD5A2) gene encodes for a key enzyme catalyzing the intra-prostatic conversion of testosterone to a more potent 5-alpha-dihydrotestosterone. Recently, A49T substitution of the SRD5A2 was described, leading to a variant enzyme with a Vmax five times that of the wild type. A49T was shown to be strongly associated with prostate cancer. Here, we performed a large-scale population-based study of 563 prostate cancer patients to explore the significance of the A49T variant. We also used PCR-SSCP to screen for novel SRD5A2 germline alterations in 50 prostate cancer patients. The A49T substitution was found in 6.0% of the 516 population controls, in 5.7% of 472 unselected prostate cancer patients (RR=0.97, 95% CI 0.73-1.29, P=0.89), and in 5.5% of patients with hereditary prostate cancer (RR=0.92, 95% CI 0.40-2.13, P=1.00). Therefore, in contrast to the previous report, no association of the A49T variant with prostate cancer could be demonstrated. In the PCR-SSCP analysis, no significant novel coding region mutations or SNPs were detected among 20 patients with a strong positive family history of prostate cancer, nor among 8 patients who developed prostate cancer during treatment of benign prostate cancer with finasteride. Therefore, our studies do not support the significance of genetic variation at the SRD5A2 gene locus in prostate cancer causation.

Detection of Single-nucleotide Polymorphism and Expression Analysis using Microspheres Encoded with Quantum Dot Semiconductor Nanocrystals. *E.Y. Wong¹, T.T. Le¹, M.P. Bruchez¹, V.E. Phillips¹, Y. Jiang², G.C. Gooden², J.A. Treadway¹, J.P. Larson¹, J.H. Lai¹, M.L. Bittner².* 1) Quantum Dot Corporation, Palo Alto, CA 94303; 2) Cancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Semiconductor nanocrystals (Qdot Nanocrystals) represent a new class of multi-color, highly fluorescent materials that circumvent many problems associated with the organic dyes used in biological applications. The physical and optical characteristics of nanocrystals such as high sensitivity, low cross talk and single wavelength excitation made them an ideal for use in encoding microsphere (Qbead microspheres). We have developed a method using Qdot nanocrystals to encode microspheres. These Qbead microspheres are functionalized for biological applications. We will present detection of single nucleotide polymorphisms and expression analysis using molecular beacons immobilized on Qbead microspheres.

Measuring Mutation Load in Human Cells From Paraffin Embedded Human Tissues. *S.S. Sommer, E. Heinmoller, G. Schlake, X. Li, K. Hill, Q. Liu.* Dept Molecular Genetics, City of Hope Natl Medical Ctr, Duarte, CA.

Somatic mutations can compromise the integrity of the human genome. If one could find and analyze gene mutations within single cells, individuals with a high mutation load (i.e. high mutation frequency) in normal tissues could be identified. These individuals may be at increase for cancer. Since the pattern of mutation can vary dramatically with the type of mutagenic insult or the defective repair system, the pattern of mutation elucidated by the mutation load assay can serve as a clue for identifying the nature of the causative factor. The p53 gene was used as a model, because it has two advantageous properties for the immunohistochemical assay of mutation load: i.) p53 is stabilized by missense mutation in exons 5 through 9; and ii.) mutations in the p53 gene can alter normal transcriptional regulation, thereby distinguishing cells with mutant p53 gene from the background of cells with stabilized wildtype p53 gene. To measure mutation load, slides from ethanol fixed, paraffin embedded cells were immunostained with p53 and PCNA antibodies. Single cells that stain positively for p53 with altered PCNA levels were microdissected and DNA amplified by PCR. Specific mutations were then identified by sequence analysis of exons 5 to 9. A robust method requires reproducible double immunohistochemical staining, efficient microdissection of single cells under conditions that preserve the integrity of genomic DNA, and the efficient amplification of the p53 gene from single cells (70% of efficiency). These milestones have been achieved and point mutations were detected in single cell from normal colon.

Different frequencies of mutation types in HNPCC versus HNPCC-suspected families. *R.M.W. Hofstra¹, Y. Wu¹, M.J.W. Berends², R.G.J. Mensink¹, R.H. Sijmons¹, A.G.J. van der Zee³, J.H. Kleibeuker², C.H.C.M. Buys¹.* 1) Medical Genetics, Univ Groningen, Groningen, Netherlands; 2) Gastroenterology, Univ Groningen, Groningen, Netherlands; 3) Gynaecology, Univ Groningen, Groningen, Netherlands.

Current data in HNPCC families indicate that MMR gene mutation carriers have a cumulative lifetime risk of 80% of colorectal cancer (CRC), 50% risk of endometrial cancer, and a substantially increased risk for a number of other tumors. Germline mutations in MSH2, MLH1 or MSH6 occur in 50-75% of HNPCC families fulfilling the Amsterdam criteria. In families not completely fulfilling the Amsterdam criteria (HNPCC-suspected) the detection rate of germline MMR gene mutations drops to less than 30%. To investigate whether the type of MMR gene germline mutations differs between HNPCC and HNPCC-suspected families, we screened 32 HNPCC and 199 HNPCC-suspected families for mutations in MLH1, MSH2 and MSH6. In the 32 HNPCC families we detected 15 truncating mutations and 4 possibly causative missense mutations or small in-frame insertions or deletions. In the HNPCC-suspected group we identified 15 truncating and 13 possibly causative mutations. We tentatively postulate that these missense and small in-frame deletion/insertion mutations may have a lower penetrance, and are therefore found more often in the HNPCC-suspected group.

Genotype-phenotype analysis of endometrial cancer in sardinian patients: fine mapping of a new minimal LOH region at chromosome 10q25-q26. *G. Palmieri¹, A. Manca¹, A. Cossu², P. Baldinu¹, R. Muresu¹, S. Tore¹, M. Pisano¹, M. Casula¹, G. Ruiu³, S. Dessole³, A. Pintus², G. Massarelli², F. Tanda², M. Pirastu¹.* 1) Istituto Genetica Molecolare CNR, Alghero (SS), Italy; 2) Istituto di Anatomia Patologica, Università di Sassari, Italy; 3) Dipart. Ginecologia ed Ostetricia, Università di Sassari, Italy.

Large number of molecular studies have revealed loss of heterozygosity (LOH) at variable frequency within different genomic regions in endometrial cancer (EC). However, deletion mapping studies suggested the presence of tumor suppressor gene(s) involved in EC at chromosome 10q25-q26. In addition, microsatellite instability (MSI) has been demonstrated higher in EC than in other common malignancy, mostly due to defective DNA mismatch repair. To further evaluate the role of the 10q25-q26 region in EC pathogenesis, we screened EC cases from Sardinia, whose population is genetically homogeneous and helpful in defining the molecular basis of complex diseases like cancer. Paired normal and tumor samples from 157 Sardinian patients with EC at various stages of disease were screened by PCR-based analysis using microsatellite markers spanning about 14-cM at 10q25-q26. Three subsets of tumors were identified: one, negative for genetic alterations within the 10q25-q26 region; the second, with prevalence of MSI (suggesting the presence of a "mutator" phenotype); and the third, carrying a specific allelic deletion. This latter group was further analyzed by increasing the marker density within the candidate region. Since two discreet LOH regions were previously described at 10q25-q26 in EC patients from Japan and North America, the region we identified could represent the third one. All these mapping data might be due to the presence of different tumor suppressor genes at 10q25-q26 or, more intriguing, to differences associated to patients' origin. As additional feature, we found a significant correlation between MSI and the disease stage; its role as prognostic factor is under evaluation. We have actually restricted the minimal consensus region of LOH to a genomic fragment of about 200 kb, and two candidate EST clusters are being analyzed.

Site of a putative TSG at 17p13.1 in Barrett's Oesophageal Adenocarcinoma. *J.R. Dunn¹, J. Garde¹, J.R. Gosney², B.C. Oates¹, A.J.M. Watson³, J.K. Field¹.* 1) Clinical Dental Sciences, University of Liverpool, Liverpool, UK; 2) Department of Pathology, University of Liverpool, Liverpool, UK; 3) Department of Medicine, University of Liverpool, Liverpool, UK.

We have previously identified thirteen common minimally deleted regions (MRs I-XIII) on chromosome 17 in twelve Barrett's Oesophageal Adenocarcinoma (BOA) specimens using 41 precisely mapped microsatellite markers. The aim of the present study has been to identify the earliest sites of loss on this chromosome that arise and persist during the progression to BOA. This has been undertaken by the analysis of multiple carefully microdissected tissue samples from each of five oesophagectomy specimens, several of which contained identifiable pre-malignant tissue. An LOH profile was constructed for each specimen using loss data generated from tissue histotypes ranging from normal epithelium to adenocarcinoma. Our data demonstrates a stepwise accumulation of loss in each specimen, consistent with a single clonal pathway in four specimens and several co-existing pathways in one specimen. Within extensively deleted regions of the tumour (seen in three specimens), small deletions were detected in pre-malignant tissue predominantly at the site of our identified minimal regions, and these losses were seen to expand and merge during the progression to BOA. Clonal losses at minimal regions were first detected in tissue showing early changes histologically, including Barrett's intestinal metaplasia and intermediate grade dysplasia. In particular, LOH at minimal region III (MRIII) occurred in the earliest tissue in all five specimens. MRIII is a novel region situated at 17p13.1 and is approximately 500 kb in size. We have confirmed that MRIII is separate from p53 and now propose to further investigate this region as the site of a putative tumour suppressor gene involved in BOA. This work was sponsored by the North West Cancer Research Fund.

Gene expression profile of malignant mesothelioma cell lines using cDNA microarrays. *S. Apostolou¹, A. De Rienzo¹, S.S. Murthy¹, C. Patriotis², J.R. Testa¹.* 1) Human Genetics, Fox Chase Cancer Center, Philadelphia, PA; 2) Molecular Oncology, Fox Chase Cancer Center, Philadelphia, PA.

Malignant mesothelioma (MM) is a highly aggressive mesodermal neoplasm characterized by a long latency following exposure to asbestos. The length of the latent period suggests that multiple somatic genetic alterations may be required for tumorigenic conversion of a mesothelial cell. To identify genes relevant to MM, we compared normal mesothelial cells with 4 MM cell lines using Atlas human cancer cDNA array membranes (CLONTECH). We identified 6 upregulated and 8 downregulated genes common to all 4 MM cell lines relative to the normal mesothelial cells. Most of these genes are located in chromosomal regions previously shown by comparative genomic hybridization to be gained or lost, respectively, in MM. Several of the overexpressed genes encoded products involved in DNA replication control and apoptosis, rho GDP dissociation inhibitors, and intermediate filament markers. The underexpressed genes encoded receptors, cell adhesion molecules, cytokines, and invasion regulators. The downregulated procollagen alpha subunit precursors, COL1A2 and COL3A1, and the metalloproteinase inhibitor precursors, TIMP1 and TIMP3 have not previously been shown to be involved in the pathogenesis of MM. In addition, the upregulated, rho GDP dissociation inhibitor, ARHGDIA, has not been implicated in this malignancy. RT-PCR is underway to verify the expression levels of the up- and down-regulated genes. If differential expression is confirmed, the genes will be further characterized to determine their role in MM. *COL1A2 COL3A1 TIMP1 TIMP3 ARHGDIA.*

Expression screening to identify target genes of chromosomal alterations: cDNA microarray reveals a novel amplified, overexpressed and rearranged gene MB-17A at 17q23 in breast cancer. *O.M. Monni¹, M. Barlund², J. Kononen¹, S. Mousses¹, J.D. Weaver¹, M. Bittner¹, Y. Chen¹, J. Torhorst³, G. Sauter³, O-P. Kallioniemi¹, A.*

Kallioniemi¹. 1) Cancer Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Institute of Medical Technology, University of Tampere and Tampere University Hospital, Finland; 3) Institute of Pathology, University of Basel, Switzerland.

Identification of target genes for chromosomal rearrangements by traditional methods involves substantial amount of work, especially if the regions of involvement are large. Here, we developed a new strategy to identify target genes for DNA amplifications, based on cDNA microarray analysis of all known transcripts from the region of involvement. A custom-made cDNA microarray of 615 transcripts was generated to cover all known genes from chromosome 17, as well as full regional representation of two amplicons: 17q12 (HER-2 amplicon), and 17q23-q24, another common amplification site in breast cancer. In breast cancer cell lines with 17q12 amplification, HER-2 was the most highly overexpressed gene, thereby validating this expression based screening strategy in the identification of target genes. The most highly overexpressed transcript in the 17q23 amplified cell lines was a novel EST, which was cloned and predicted to encode a 913 aa protein with no homologies to any other known proteins. High-level amplification of this MB-17A gene was verified by FISH in all breast cancer cell lines with 17q23 amplification and in 24 of 280 primary breast cancers (8.6%) based on a tissue microarray analysis. In breast cancer cell lines with 17q23 amplification, Northern blotting showed overexpression, in some cases of a structurally rearranged gene. The results suggest that MB-17A may be a genomic target for chromosomal rearrangements at 17q23 and play a critical role in breast cancer progression. In summary, with the completion of the human genome sequence, cDNA microarrays with full representation of all transcripts from a chromosomal region can be constructed and applied to systematically uncover the molecular consequences of chromosomal rearrangements in cancer.

Classification of Renal Cell Carcinoma with cDNA Microarray. *K. Zhang¹, M. Xiong¹, L. Jin¹, L. Rogers², R. Amato², A. Killary², S. Lott³.* 1) Human Genetics Center, UT-Houston, Houston, TX; 2) MD Anderson Cancer Center, University of Texas, Houston, TX; 3) NEN Life Science Products, Inc., Boston, MA.

With the ability to simultaneously monitor the expression level of thousands of genes, microarrays have become a powerful tool for tumor classification. However, the most popularly used direct labeling methods require large quantities of RNA, which are often unattainable from tissue samples collected from residual surgical specimens. To overcome this obstacle, we utilized NENs MICROMAX TSA (tyramide signal amplification) microarray system, from which we were able to measure gene expression profiles from only 100 mg of available tissue. Twenty-two pairs of renal cell carcinoma and adjacent normal kidney tissues were collected from patients undergoing primary nephrectomy with no prior history of chemo/immunotherapy. Expression levels of 2400 genes were simultaneously measured using labeled cDNA and TSA detection. A novel application of Fishers linear discriminant analysis was used to rank genes according to their ability to classify tumor and normal tissues. Candidate genes for classification were then selected based on their ranking scores. Our preliminary data based on ten pairs of samples suggests that, with the expression profile of only 26 genes, the tissue samples can be perfectly classified by a hierarchical clustering method. Several of these genes are known to have a biological function during tumor initiation and progression, such as the VHL binding protein and 14-3-3 epsilon. Additional genes that have possible functions in transcription regulation and cell growth regulation were identified, including HLH basic phosphoprotein(G0S8) and Cellular growth-regulating protein (L10844). In comparison, classification with expression profiles of all genes leads to poor cluster results and no genes with biological significance could be easily identified. A comprehensive analysis using the full set of gene expression data, in combination with the patients clinical information, is in progress to confirm these results.

Defining the unique expression pattern for Barrett's-associated oesophageal adenocarcinoma (BA) by comparing pooled normal versus disease oligonucleotide array data. *A.J. Williams¹, J.F. Boland¹, R.V. Lord², C. Alvares¹, J.C. Wetzel¹, U. Scherf¹, J.G. Vockley¹.* 1) Gene Logic INC., Gaithersburg, MD 20878; 2) Department of Surgery, University of Southern California Keck School of Medicine, Los Angeles, CA 90033.

To fully understand the genetic basis of a given disease, it is necessary to identify all essential genes that reliably exhibit abnormal expression values in a wide variety of patients. We have refined a strategy that systematically examines normal and diseased tissue samples to determine the patterns of gene expression that are unique to disease. This approach was tested by performing oligonucleotide array studies and pooling the data for individual normal oesophagus and Barrett's-associated oesophageal adenocarcinoma (BA) samples. We first identified all genes from the normal or diseased samples that were either commonly expressed or commonly not expressed. Next, we revealed the unique pattern of genes over-expressed in the disease by determining those genes that were expressed in BA, but not expressed in normal oesophagus. Similarly, the unique pattern of genes under-expressed in disease was found by identifying genes that were expressed in normal oesophagus, but not expressed in BA. These results were combined with fold change analysis to rapidly discover a subset of key disease-related genes. The identified genes were used as signposts pointing to significant gene clusters in hierarchical clustering analysis. Our approach should be useful for the relatively quick and systematic identification of potential therapeutic and diagnostic targets for a wide variety of diseases.

Somatic and/or germline mosaicism for recurrent APC gene mutations. *E. Bakker, E.C. Bik, Y.M.C. Hendriks, R.H.A.M. Vossen, C.M.J. Tops.* Department of Human and Clinical Genetics, Leiden University Medical Center, Wassenaarseweg 72, 2333AL Leiden, Netherlands.

Familial adenomatous polyposis (FAP [MIM 175100]) is an autosomal dominant disorder caused by germ-line mutations in the APC gene (M74088 [GenBank]). Scanning of the APC gene in 530 Dutch FAP and 'FAP-like' patients has resulted in the identification of 130 pathogenic mutations. These mutations are scattered along a large part of the gene, with some recurrent mutations. Somatic mosaicism was evident in 5 of the 130 Dutch pathogenic mutations, with an apparent preference for a nonsense mutation in exon 8. The presence of low (10-20%) mosaicism of the exon 8 mutation was detected by DGGE and/or SSCP scanning of sporadic cases of clinical evident poliposis coli. In one family there was a woman (85 years, healthy) with an apparent isolated gonadal mosaicism, since the mutation was transmitted twice to the next generation on the maternal haplotype, whereas the mutation was not detectable in the maternal lymphocytes or skin-fibroblasts. Since the APC de novo mutation rate is estimated to be in the order of 25% (Bisgaard et al. Hum Mutat, 1994, 3:121-5), germline and/or somatic mosaicism could account for a substantial portion of the solitary FAP or 'FAP-like' cases and a mutation scanning directed towards identifying a low-abundant mutated APC allele should be considered in the non-familial cases. New detection techniques like pyrosequencing (Ahmadian et al. Anal Biochem, 2000, 10:103-10) are currently evaluated as a method to detect low level mosaics.

Association of the aromatase gene (Cyp19) and endometrial cancer -- Testing for Hardy-Weinberg

disequilibrium in cancer patients. *C.C. Gu¹, D.G. Mutch², P.J. Goodfellow³*. 1) Div Biostatistics,; 2) Dept. of Obstetrics and Gynecology; 3) Dept. of Surgery, Washington Univ Sch Medicine, St Louis, MO.

Estrogen is a potent risk factor for the development of a variety of cancers, including endometrial carcinoma. Siegelmann-Danieli and Buetow (S&B, 1999) recently reported an association between constitutional variation in the aromatase (estrogen synthetase) gene, CYP19, and breast cancer risk. In a case-control study, they investigated an intron 4 tetranucleotide repeat polymorphism and saw an over-representation of a specific allele in breast cancer cases. We undertook a study to determine whether CYP19 variation is associated with endometrial cancer risk. We genotyped 222 endometrial cancer patients for the intron 4 tetranucleotide repeat marker and assessed marker-disease association by testing for Hardy-Weinberg disequilibrium (HWD) in cancer cases. The overall allelic distribution in our cases is similar to that reported by S&B (1999) in breast cancer patients, although in our study the distribution in white patients is significantly different from that in blacks. We carried out analyses of HWD in patient subgroups stratified according to ethnicity, age at cancer presentation and tumor histologic subtype. The average age of onset differs by 5 years between patients with type I (endometrioid) and type II (nonendometrioid) cancers. In the type I cases, HWD is detected by Fisher's exact test with a p-value of 0.03 (N=18) in blacks (p=0.24 in whites with N=166). Tests for HWD in type II cases were nonsignificant in both ethnic groups (p=0.143 in whites with N=22; p=0.90 in blacks with N=16). These preliminary results suggest that CYP19 variation may be a contributing risk factor in type I endometrial cancer patients. We found that while the high-risk allele (171bp) reported by S&B (1999) is more frequent in cases of younger age of onset (<55 yr.), the test for HWD is nonsignificant in this subgroup. A case-control study extending findings reported here is underway to further define the role of aromatase and to quantify contributions of constitutional genetic variation of other estrogen-related genes to the development of endometrial cancer.

A genome-wide scan of 94 prostate cancer families stratified by clinical features identifies loci of interest. *E.A. Ostrander¹, E.L. Goode², S. Kolb¹, M. Janer³, M. Badzioch², M. Peters¹, M. Gibbs¹, J.L. Stanford¹, G.P. Jarvik², L. Hood^{2,3}.* 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) University of Washington, Seattle, WA; 3) Institute for Systems Biology, Seattle WA.

A 10 cM genome-wide scan of 94 prostate cancer families, including 432 affected men, is being analyzed to identify prostate cancer susceptibility loci. Fifty families are classified as early-onset (median age at diagnosis <66 years); 44 as later-onset (> or = 66 years) and 11 as earliest onset (<61 years). When the entire data set was considered previously, regions of interest were identified on chromosomes 8, 10, 12, and 14. Stratification by tumor stage and grade provide additional support for findings on 8, 10 and 14, and highlights additional regions of interest on chromosomes 6, 20 and X. GENEHUNTER was used to compute multipoint NPL scores for linkage in the stratified data sets. We observe an NPL score of 2.58 ($p = 0.014$) for marker D10S1213 in families in the <61 group with local/regional disease, and an NPL of 2.24 ($p = 0.026$) at D14S587 in the <66 group in families with at least one member with advanced stage disease. We also observe an NPL of 2.82 ($p = 0.006$) at D8S1130 in later-onset families with at least one case with high grade disease. For chromosomes 8 and 10, multipoint Lod scores >2.0 were observed in the same regions in our initial genome scan of all 94 families. An additional region of interest in the stratified screen is identified by an NPL of 2.55, ($p = 0.015$) at D6S474 in the <61 families who have at least one prostate case with advanced stage disease, suggesting a locus that predisposes to a more severe form of familial prostate cancer. Finally, we observed significant results at two previously described loci: D20S478 (NPL = 3.32, $p = .003$) in the HPC20 region in families > or = 66 with at least one case of advanced stage disease, and at the HPCX locus with marker DXS984 (NPL = 2.11, $p = 0.023$) in families with local/regional disease. In aggregate, these data suggest the existence of several prostate cancer susceptibility loci, and that analysis of data by a variety of criteria is needed to fully understand genetic susceptibility to the disease.

An exploration of the role of family history of cancer among four rare types of cancer. *H.H. McDuffie, P. Pahwa, S. Fincham, J.J. Spinelli, J.R. McLaughlin, J.A. Dosman, D. Robson.* Ctr Agric Med, Royal Univ Hosp, Univ Saskatchewan, Saskatoon, SK., Canada.

A positive family history of cancer can reflect both environmental exposures and genetic susceptibility. Hodgkin's Disease (HD), Multiple Myeloma (MM), non-Hodgkin's Lymphoma (NHL) and Soft Tissue Sarcoma (STS) have several putative etiologies and risk factors in common. The risk factors include a positive history of cancer among first degree relatives, perturbed immune function, occupational exposure to specific pesticides, to viruses and chromosomal instability. Simultaneously and using identical methodology, we obtained cancer history information on the first degree relatives of male incident cases (316 HD, 347 MM, 517 NHL, 365 STS and 1506 sex, age 2 year and province of residence matched controls using postal questionnaires. Family history of cancer included relationship to the proband, the types of tumours diagnosed among relatives, the year and province of diagnosis. We conducted conditional logistic regression analyses adjusted for age and province of residence and report adjusted odds ratios (OR) and 95% confidence intervals (95% CI). Probandes were excluded from all statistical analyses. We found (a) that most families are cancer free (excluding probands)(range 52% MM to 67%; controls); (b) few families have two or more affected first degree relatives (range 5.7% HD to 18.7% MM);(c) HD cases had fewer siblings and children compared to other case groups and to controls;(d) adjusted for family size, HD, MM and NHL families have significantly higher risks of affected siblings compared to control families;(e) among all cases and controls; cancers of the trachea, bronchus, lung and of the female breast are first or second in frequency among relatives. Lymphoid leukemia ranked third or fourth in frequency among relatives of HD, NHL and STS cases; (f) leukemia, lymphoma and multiple myeloma occur in excess in families of HD cases (OR (95% CI) 3.61 (1.65, 7.86) compared to controls adjusted for pesticide exposure. Conclusions:(a)a positive family history of cancer is an independent risk factor for HD,MM and NHL. b)the evidence is strongest for HD, followed by MM and NHL.

Effect of stratifying by linkage at known loci in assessing a genome-wide scan of familial prostate cancer. *M.D. Badzioch¹, J.L. Stanford², M. Gibbs², M. Janer³, E.L. Goode¹, M. Peters², S. Kolb², L. Hood³, G.P. Jarvik¹, E.A. Ostrander².* 1) Division of Medical Genetics, University of Washington; 2) Fred Hutchinson Cancer Research Center; 3) Institute for Systems Biology, Seattle, WA.

Five putative familial prostate cancer (FPC) high-risk loci, HPC1, PCAP, HPCX, CAPB, and recently, HPC20 (chr. 20q13) have been reported to date. To increase genetic homogeneity of analysis groups, most genome scan studies stratify by average age at diagnosis, number and distribution of affected individuals, and clinical characteristics, but linkages to putative high-risk loci have not yet been used for this purpose. We present the effects of stratifying by multipoint NPL scores at putative high-risk loci in assessing a 10 cM scan of 94 FPC families. Classified by age alone, the highest linkage scores were at CAPB (NPL=2.28, avg. age at diagnosis <61 years) and on chrs. 4 (NPL=2.06, <66), 8 (NPL=2.02, ³66), and 15 (NPL=2.04, ³66). By HOMOGM, we estimated the percentages of families showing some evidence of linkage to the first 4 candidate regions to be 12.5, 10.3, 7.1, and 18.2, respectively, leaving 51.9% without such evidence. We split all families into 2 groups of roughly equal size by assuming true linkage to any of the 4 regions if an NPL score was ³0.7. We then resurveyed the genome using sets of families defined by median age of diagnosis and prior linkage status. The highest NPL scores now occurred chr. 4 (NPL=2.68, p=0.006) in early-onset families having no evidence of prior linkage. In the subset with evidence of prior linkage, peaks were found on chr.10 (NPL=2.49, p=0.014) in early-onset and on chr. 20 (NPL=2.76, p=0.005) in late-onset FPC. Since the result on chr. 20 was consistent with HPC20 we maximized the NPL score to 4.20 by increasing the cutoff NPL at other loci to ³1.10. These results suggest that prior linkages, in addition to clinical and family data, may identify more homogenous subgroups for scan analysis. Although caution should be taken when testing multiple strata, the fifth putative FPC locus, HPC20, appeared in this dataset after partitioning on prior linkages. These results support the existence of multiple FPC loci and suggest the possibility of locus interaction.

CYP17 promoter polymorphism and breast cancer in Australian women under forty years of age. *A.B. Spurdle¹, X. Chen¹, J. Hopper², D. Easton⁵, G. Dite², J. Cui², M. McCredie³, M. Southey⁴, D. Venter⁴, G. Chenevix-Trench¹.* 1) Queensland Inst Med Res, Brisbane, Queensland, Australia; 2) University of Melbourne, Victoria; 3) New South Wales Cancer Council, Sydney; 4) Peter MacCallum Cancer Institute, Melbourne; 5) Strangeways Research Laboratory, Cambridge, UK.

We conducted a population-based, case-control-family study to assess the relationship between the cytochrome P450c17a (CYP17) 5' promotor region T to C polymorphism and early-onset breast cancer. CYP17 genotype was determined in 369 cases under 40 years, 284 controls and 91 relatives. Genotype distributions were compared by logistic regression, and cumulative risk estimated by a modified segregation analysis. The TC genotype was not associated with risk ($P = .7$). Compared to TT and TC, the CC genotype was associated with a relative risk (95% confidence interval) of 1.81 (1.15-2.86) before, and 1.63 (1.00-2.64) after adjustment ($P = .01$ and $.05$, respectively). There was an excess of CC genotypes in cases with at least one affected first- or second-degree relative (23% compared with 11% in controls; $P = .002$), and these cases were at a 3- to 4-fold risk compared with other groups. Analysis of breast cancer in first- and second-degree relatives of CC cases, excluding two known carriers of a deleterious mutation in BRCA1 or BRCA2, gave a relative hazard in CC women of 3.48 (1.13-10.74) ($P = 0.04$), equivalent to a cumulative risk of 16% to age 70. This finding is currently being extended to a larger case-control-family study from the Cooperative Family Registry for Breast Cancer Studies of women diagnosed before and after age 40.

Detection of high-order interactions among estrogen metabolism genes in sporadic breast cancer. *M.D. Ritchie, N. Roodi, L.R. Bailey, W.D. Dupont, W.D. Plummer, L.W. Hahn, F.F. Parl, J.H. Moore.* Program in Human Genetics, Vanderbilt University, Nashville, TN.

Mammary metabolism of estrogen to carcinogenic catechol estrogens is thought to increase the risk of sporadic breast cancer. An important question is whether breast cancer risk can be predicted by knowledge about variation in genes from the estrogen metabolism pathway. Previous studies by our group and others suggest that genes from this pathway do not have main or independent effects. The goal of this study was to determine whether interactions among variations in genes from this pathway are associated with sporadic breast cancer.

Our sample included 414 age-matched Caucasian women: 207 cases and 207 controls. We measured eight polymorphisms in five genes from the estrogen metabolism pathway: *CYP1A1*, *CYP1B1*, *COMT*, *GSTM1*, and *GSTT1*. We employed a multifactor data reduction method to evaluate each of the possible interactions among the eight polymorphisms. For each particular combination of loci, we identified multilocus genotypes that are more common in cases than controls and pooled these into a single group. This reduces the dimensionality of the data to one. We estimated the prediction error or misclassification rate of each combination of loci and their optimal multilocus genotype grouping using nine-fold cross validation.

The best model included five polymorphisms, one from each of the five estrogen metabolism genes. Permutation testing indicated the probability of observing the misclassification rate for this model, given the null hypothesis of no association, was less than 0.05. This study illustrates the importance of considering high-order genetic interactions in studies of complex diseases such as sporadic breast cancer when there are no main or independent genetic effects.

Denaturing high performance liquid chromatography (DHPLC) used in the detection of mutation in hMLH1 and hMSH2 genes. *S. Pawar*¹, *S. Ciocchi*², *A. Markowitz*², *S. Jhanwar*², *N. Ellis*². 1) IMPATH, New York, NY; 2) Memorial Sloan-Kettering Cancer Center New York, NY.

Hereditary nonpolyposis colorectal cancer (HNPCC) is the most common genetic condition that determines susceptibility to colorectal cancer. Studies for this disease have revealed it to be a simple mendelian disease involving five mismatch repair genes: hMLH1 and hMSH2 being the major ones. Rapid and accurate identification of DNA sequence heterogeneity is being recognized as of major importance in disease management. In this study we have used Denaturing high performance liquid chromatography (DHPLC) as a method of screening for germ line mutation in the hMSH2 and hMLH1 genes. The method is based on differential separation and detection of mismatched heteroduplexes formed after re-annealing of normal and mutant DNA strands. Analysis of all the exons and the exon-intron borders of hMSH2 and hMLH1 genes in 16 unrelated HNPCC kindreds was done. Optimization of DHPLC analysis of each exon was carried out by design of primers with minimum variation in the melting temperature of the amplicon, and titration of both elution gradient and temperature. To confirm the validity of DHPLC analysis all the 560 amplicons corresponding to 16 fragments for hMSH2 gene and 19 fragments for hMLH1 gene for each DNA sample were directly sequenced. All but 2 patients tested showed at least one heterozygous DHPLC profile in either hMLH1 or hMSH2 gene. A total of 18 unique DNA sequence variants were identified. Sequencing of the variant amplicons detected one novel and four previously reported pathogenic mutations, as well as seven novel and six known polymorphisms or unique sequence variants that are probably of no clinical significance. The amplicons that were observed to have a normal homozygous DHPLC profile detected no sequence variation in cycle sequencing. Therefore under our DHPLC conditions 100% of the sequence variation in hMLH1 and hMSH2 genes for the 16 individuals were correctly identified. Our results demonstrate that DHPLC is a highly sensitive, specific and inexpensive method to screen for mutations.

Caveolin-1 gene promoter methylation in prostate cancer. *J. Cui¹, L.R. Rhor², G. Swanson³, V.O. Speights⁴, M. Powers¹, T.M. Maxwell¹, A.R. Brothman¹.* 1) Dept. of Pediatrics and Human Genetics, University of Utah, Salt Lake City, UT; 2) Dept. of Pathology, University of Utah, Salt Lake City, UT; 3) Cancer Care Northwest, Spokane, WA; 4) Dept. of Pathology, Scott & White Hospital, Texas A & M University, Temple, TX.

Hypermethylation of CpG islands in the promoter regions of tumor suppressor genes appears to be a common mechanism of tumorigenesis. Caveolin-1 (Cav-1), a gene coding for the structural component of cellular caveolae, is involved in cell signaling and has been proposed as a tumor suppressor gene in several malignancies. We analyzed the methylation status of CpG sites in the Cav-1 promoter region on 26 primary prostate tumor samples. Genomic DNA from both tumor and normal specimens was obtained from paraffin-embedded prostate sections by laser capture microdissection (LCM) and was bisulfite modified and PCR amplified to evaluate 24 CpG sites at the 5' promoter region of exon 1 of Cav-1 by bisulfite genomic sequencing. Immunohistochemistry staining with a caveolin-1 specific antibody was also performed to evaluate the expression of the gene. Twenty out of the 22 (91%) informative cases showed promoter hypermethylation in the tumor cell population when compared with adjacent normal prostate cells ($p=0.0001$). An elevated immunoreactivity was observed in tumor cells from 7 of 22 prostate samples tested but this did not correlate with Gleason grade or PSA failure. While no association with Gleason grade was found, overall increased methylation correlated with PSA failure ($p=0.016$), which may predict clinical recurrence of the disease. These findings support the notion that Caveolin-1 functions as a tumor suppressor gene in prostate cancer and suggest that methylation status of this gene may be a marker predictive of outcome. Supported by NIH grant R01CA46269 and a grant from the University of Utah.

Overexpression of proline oxidase, a late p53-induced-apoptosis gene (PIG6), induces apoptosis in cancer cells.

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Mutations in p53 are a major contributor to malignancy and have profound effects on cell cycle regulation, DNA repair and apoptosis. In particular, cells lacking p53 have defective apoptosis but the role played by p53 in this complex process is only now being elucidated. In a SAGE study of p53-induced apoptosis in a colorectal cancer cell line (DLD-1), the expression of only 14 of 72,02 genes (0.19%) was found to be induced >10X. Of these, 3 were early (~3 hr) and 11 were late (>12 hr). One of the late p53-induced genes, proline oxidase (POX), a mitochondrial inner-membrane protein catalyzes the oxidation of proline to pyrroline-5-carboxylate. Expression of POX was increased 12-fold following p53 overexpression. A number of aspects of POX make it an interesting candidate for playing a role in apoptosis. These include its location in the inner-mitochondrial membrane; the potential for the POX reaction to influence the intramitochondrial redox ratio; and the use by POX of cytochrome C (cyt. C) as an electron acceptor. To study the possible role of POX in apoptosis, we established several DLD-1 lines harboring an inducible POX gene using the tet-off system. We showed that DLD-1 cells expressing high POX activity undergo a proline-dependent apoptotic response. The effect is apparent at 0.5 mM and maximal at 5 mM proline. Normal plasma proline concentrations (mean 160 mM; range 50 to 280 mM) approach these concentrations. Under induction conditions, apoptosis of the DLD-1 cells peaks at six days with massive cell death associated with release of cyt. C from mitochondria, caspase-9 activation, and chromatin condensation. Additionally, ROS in the POX-overexpressing cells were increased by 150 to 200% above controls. We conclude that POX induction in the presence of proline is sufficient to induce classical apoptosis perhaps through generation of ROS or alteration of cyt. C. The relation of these observations to the in vivo role of p53 in apoptosis remains to be determined.

Molecular and Cellular Pathology of Benign Neurofibromas. *E. Serra¹, E. Ars¹, M. Nadal¹, A. Sanchez², A. Ravella³, T. Rosenbaum⁴, X. Estivill¹, C. Lazaro¹.* 1) Medical and Molecular Genetics, IRO, Hospitalet Llob, Barcelona, Spain; 2) Genetics Service, Hospital Clinic, Barcelona, Spain; 3) Dermatology Service, Hospital Creu Roja, Barcelona, Spain; 4) Department of Neuropediatrics, Heinrich-Heine-University, Dusseldorf, Germany.

Neurofibromas, benign tumours that originate from the peripheral nerve sheath, represent a hallmark of Neurofibromatosis type 1 (NF1). Although their unicellular origin has been confirmed they are difficult to study due to their multicellular composition. Several studies suggested and our group recently demonstrated that Schwann cells are the NF1(-/-) cells in this type of tumour. Although different LOH studies indicated that inactivation of both copies of the NF1 gene is a common feature in neurofibromas, the presence of the two hits in the NF1 gene has only been reported in few neurofibromas. In this work our group presents the widest mutational study of the NF1 gene in a large number of neurofibromas by using several methods including SSCP/HD analysis and LOH studies. In total we have identified 42 somatic NF1 mutations from a pool of 123 neurofibromas. LOH is presented in 25% of the studied neurofibromas. We differentiate two types of LOH: one being true deletions of part or the whole NF1 gene, and a second type of LOH affecting the whole long arm of chromosome 17, which arose through a mitotic recombination event. By cDNA-SSCP/HD analysis we have detected 13 NF1 somatic point mutations, being nonsense mutations, small deletions, mutations in the consensus splice site or nonsense mutations producing exon skipping, and mutations causing the introduction of cryptic exons. As we have previously demonstrated in the study of germline NF1 mutations somatic point mutations causing splicing alterations are also common. The analysis of various neurofibromas from patients with sporadic NF1 can facilitate the identification of their germline mutation. The demonstration that somatic and germline mutations are in the two NF1 copies, corroborates the "double hit" model for the NF1 gene in the generation of these benign neoplasias, and also shows that both mutated alleles can be expressed in neurofibroma tumoural cells.

Molecular genetic analysis on the BRCA1 gene in thirty Chilean families with breast cancer. *M. Gallardo¹, L. Egana¹, D. Austin¹, H. Paredes², M. Rodriguez³, T. Walsh⁴, K. Gordon⁴, C. Rousseau⁴, M-C. King⁴, M.P. Carvallo¹.* 1) Depto de Biologia Celular y Molecular, Fac.Cs.Biologicas,P.Universidad Catolica de Chile, and (formerly) Facultad de Medicina, Universidad de Chile, Santiago, CHILE; 2) Instituto Nacional del Cancer, Santiago,CHILE; 3) Hospital Barros Luco, Santiago, CHILE; 4) University of Washington, Seattle,USA.

Several studies involving the molecular analysis of BRCA1 and BRCA2 genes in families affected of breast cancer, from worldwide populations, have been developed. The results describe a variable frequency for BRCA1 and BRCA2 mutations, depending on the ethnic origin of the population analyzed. Thirty Chilean families were evaluated for inherited mutations in BRCA1. Families were selected by any of the following criteria: three cases of breast cancer in first degree relatives; or two cases of breast cancer in first degree relatives, one diagnosed before age 40; or one breast cancer and one ovarian cancer in first degree relatives. The 23 exons encoding the BRCA1 gene were PCR amplified and analyzed by SSCP and DNA sequencing. Two mutations were identified, both novel. Family 5 includes mother, daughter and maternal cousin diagnosed with breast cancer at ages 67, 43, and 37, respectively. Frameshift mutation 308insA was identified in BRCA1 exon 5 in the surviving affected mother and daughter. An unaffected 69-year-old aunt is an obligate carrier of the mutation. Family 14 includes 7 cases of breast cancer, 5 cases of prostatic cancer, 1 case of uterine cancer, and 1 case of stomach cancer. Nonsense mutation 3936 C->T, causing a stop at codon 1273, was identified in BRCA1 exon 11. In addition to the breast cancer cases, at least 3 of the prostatic cancer cases and both women with uterine and stomach cancer, are obligate carriers of the mutation. In order to find mutations in the remaining families, we will test BRCA1 with additional approaches, such as PTT and direct sequencing of some exons, and also screen BRCA2. It is of particular interest to know whether BRCA1 and BRCA2 mutations may manifest as cancers other than breast or ovary in Chilean families. Supported by NIH-FIRCA grant R03 TW008.

Analysis of p16 protein expression in familial melanoma patients: correlation with germline status. *P. Ghiorzo¹, B. Villaggio², N. Hayward³, G. Bianchi-Scarra¹*. 1) Department of Oncology, Biology & Genetics, Univ Genova, Genova, Italy; 2) Department of Internal Medicine, Univ Genova, Genova, Italy; 3) Queensland Institute of Medical Research, Brisbane, Australia.

The CDKN2A gene, which encodes the p16^{ink4} cell-cycle inhibitor, is the major melanoma suppressor gene. It is frequently deleted, mutated or rearranged in sporadic melanoma and in the germline of approximately 20 to 40% of familial melanoma patients. Its location on 9p21, a chromosomal region whose cytogenetic and molecular alterations correlate with clinical stages of melanoma progression, suggests that dysfunction of this gene could be critical to the evolution of melanoma. Loss of p16 expression has been found to correlate with tumor progression in sporadic melanoma, confirming this hypothesis. In this study we looked at p16 expression by immunohistochemistry in familial melanoma patients that had been characterized for p16 germline status. We collected specimens from lesions representing all stages in the progression of melanoma from patients belonging to 10 families carrying wildtype p16 and mutated p16 (G101W, in 5 families). In some cases we were able to analyse multiple lesions from the same patient. To improve interpretation of results and avoid confounding nonspecific signal, we performed the analysis with 3 different antibodies. Results of digital image analysis revealed correlation of a progressive loss of p16 expression with melanoma progression, and in some cases p16 loss was observed at an early stage of tumor progression. Moreover, the pattern of p16 localization, which was both nuclear and cytoplasmic in lesions from p16 wildtype patients, was predominantly nuclear in G101W (p16-mutated) patients, suggesting that a feature of the mutant protein is that it affects intracellular localization. This would confirm in vivo what has been previously observed in functional re-assessment of this p16 variant, which also detected a predominantly nuclear localization of the mutant protein by immunofluorescence.

Are Mutations in the von Hippel Lindau Gene Involved in Renal Carcinogenesis in Tuberous Sclerosis Complex Patients? *K.A. Duffy¹, T. Al-Saleem², E. Petri Henske¹*. 1) Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA; 2) Department of Pathology, Fox Chase Cancer Center, Philadelphia,.

Tuberous sclerosis complex (TSC) is a tumor suppressor gene disorder characterized by seizures, mental retardation, and tumors of the brain, heart, skin, and kidney. The cellular pathways leading to renal cancer in TSC patients have not been characterized. Renal cell carcinomas occur in children as well as in adults with TSC and are pathologically heterogeneous, including clear cell, chromophobe, and papillary subtypes. This heterogeneity is in contrast to von Hippel Lindau (VHL) patients who develop almost exclusively clear cell renal cell carcinomas. We have previously found loss of heterozygosity in the *VHL* region of chromosome 3p in some TSC renal cell carcinomas. In addition, the majority of sporadic clear cell carcinomas have *VHL* mutations. Therefore, mutations in *VHL* could be downstream genetic events in some TSC-associated renal cell carcinomas, particularly those with clear cell morphology.

We analyzed renal cell carcinomas from seven TSC patients for mutations in the *VHL* gene. All tumors were clear cell in subtype. The age range of the patients was 7-68, with an average age of 41 years. The *VHL* gene was examined by direct sequencing of tumor DNA from paraffin embedded tissues. To date, we have not identified mutations in any of the three exons of the *VHL* gene. This is, to our knowledge, the first indication that clear cell renal cell carcinomas in TSC develop in the absence of *VHL* mutations. This may have biological and clinical relevance to the cellular pathways leading to renal cancer in the general population.

Screening B cell lymphomas for mutations in the Nijmegen Breakage Syndrome gene (NBS1). *K.M. Cerosaletti^{1,2}, A. Morrison¹, D. Willerford², P. Concannon^{1,2}*. 1) Molecular Genetics, Virginia Mason Res Ctr, Seattle, WA; 2) University of Washington School of Medicine, Seattle, WA.

Nijmegen Breakage Syndrome (NBS) is a rare autosomal recessive disorder characterized by chromosomal instability, radiation sensitivity, immunodeficiency, and a high incidence of cancers. Among NBS patients with malignancies, 73% were B cell lymphomas. The gene mutated in NBS patients (NBS1) encodes a novel 85 kD protein (nibrin). All NBS patients have truncating mutations in NBS1 and do not produce full-length protein. Nibrin appears to play a role in maintaining genomic integrity, since it forms a complex with the Mre11/Rad50 proteins, involved in the repair of DNA double strand breaks, and may be the primary target of phosphorylation by ATM in response to ionizing radiation. To determine if the NBS1 gene is mutated in sporadic cases of non-Hodgkins B cell lymphoma (NHL), we screened 91 NHL cases and 154 normal controls from the Seattle area. DNA was extracted from paraffin blocks of lymphoma samples and PBL of normal controls, and was screened for mutations in the NBS1 gene by SSCP. We found one null mutation, the founder mutation 657del5, in one of the NHL DNAs (1.1% incidence, 95% CI 0.03%-6%) but not in the normal controls (0% incidence, 95% CI 0%-1.9%). One other variant was identified in a NHL DNA which altered an amino acid, 628G/T > V/F210. This variant was not found in the controls. A different variant, 643C/T > R/W215, was identified in a control. The effect of these amino acid changes on nibrin structure or function is unknown. The remaining sequence variants identified included five common polymorphisms, 102G/A, 553G/C > E/Q185, IVS9+18C/T, 1197 T/C, and 2016A/G. Five rare variants that do not alter the amino acid sequence of nibrin were also identified. The frequency of these polymorphisms did not differ significantly between cases and controls. Given the low incidence of NBS reported in North America, < 1 in 10 million, identification of a NBS1 mutation in a small sample of NHL cases suggests that carriers may be at increased risk for NHL. However, mutations in the NBS1 gene do not appear to be a major contributor to NHL susceptibility in the U.S. population.

MOLECULAR STUDY OF WT1, IGF2, H19 Y P57KIP2 GENES IN MEXICAN CHILDREN WITH WILMS' TUMOR ^{KIP2}. A. Cervantes¹, F. Salamanca-Gomez¹, C. Barrientos-Salcedo¹, R. Pealoza¹, D. Arenas¹, J. Vazquez², G. Sciordia³, J. Velazquez⁴. 1) Unit of Medical Investigation in Human Genetics, Children Hospital, Medical Center S. XXI,IMSS; 2) Department of Oncological Surgery, Children Hospital,Medical Center S XXI,IMSS; 3) Department of Pathology, Children Hospital,Medical Center S XXI,IMSS; 4) Department of Pediatric Surgery, General Hospital, Mediacal Center La Raza,IMSS.

MOLECULAR STUDY OF WT1, IGF2, H19 AND P57KIP2 GENES IN MEXICAN CHILDREN WITH WILMS TUMOR Barrientos C, Pealoza R, Arenas D, Palma V, Vzquez J, Velzquez J, Sciordia G, Salamanca F. Wilms Tumor is a kidney embryonic neoplasia, which in Mexico constitutes 5.5 percent of all childhood malignancy neoplasia. It has been associated to mutations in WT1 gene (Little, et al.,1995), and alterations in expression of genes that show genetic imprinting: H19, IGF2 and P57KIP2 (Taniguchi et al.,1995; Baird et al.,1997). We studied by PCR-SSCP and sequencing techniques, the mutations in the zinc finger region of WT1 gene in 25 Mexican children with sporadic Wilms tumor. Four different mutations were found. One of them not previously reported (insA1002 Y334Stop) was present in four blastemal cellular type tumors. Other mutation guanine for cytosine was change in intron 9 located at 160 bp upstream of beginning of 10 exon present in three tumors. The G1296C V432L mutation was present in two patients and the last one was C1041G that change H347N. The differential expression of parental alleles of H19, IGF2 and P57 KIP2 genes was also studied in four patients with Beckwith-Wiedemann syndrome and Wilms tumor by RT-PCR and CFLPs techniques using MspI for H19, ApaI for IGF2 and Cfo II for P57 KIP2. Our results indicated no expression of H19 in three patients and loss of imprinting (LOI) of IGF2 in two case and P57KIP2 of another two patients. These results show genetic heterogeneity of WT1 gene but a novel mutation highly prevalent in the Mexican population. These findings also support the role of genetic imprinting in the carcinogenic process.

P53 Gene Mutations in Russian Patients with Breast Cancer Detected by Chemical Cleavage of Mismatch. A.

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Mutations in the tumor-suppressor p53 gene are frequent and seen in most human cancers including breast cancer. A new solid phase chemical cleavage of mismatch method (CCM) involving silica beads made it possible for rapid and efficient screening of 89 different fluorescently labeled PCR products amplified from DNA samples extracted from tumors of breast cancer patients. Single tube CCM was incorporated and effectively utilized on silica beads. The potassium permanganate/tetraethylammonium chloride (KMnO₄/TEAC) and hydroxylamine (NH₂OH) reactions occur sequentially in a single tube. Mutation analysis involved pairing and forming heteroduplexes with the 89 different samples and screening the p53 gene from exons five to nine with the solid phase silica beads CCM. Forty-eight signals were evident in the 89 pairs of samples. Five signals were present in exons five and nine. Ten in exon six, thirteen in exon eight and fifteen signals detected in exon seven. Of the 48 CCM signals eight mutations were confirmed and identified by sequencing. The three novel mutations detected are R213L, N200K (2 patients), and L201V (2 patients). The mutations T253I, G244C and delT256 have not been reported in breast cancer but have been recorded in other tumor organs. A putative 47bp deletion at codon position 198 was detected and never reported. A mutation H179Y previously reported in breast cancer was also detected. These mutations presumably contributed to the carcinogenesis in the breast. Forty signals were not identified as a sequence change. As no false positive signals have been detected with CCM, these signals indicated CCM is more sensitive in detecting mutations in mixed mutant and wild type compared to sequencing.

Intronic *BRCA1* mutations in two highly affected kindreds. *I.M. Kedar-Barnes*¹, *P. Devilee*², *H. Meijers-Heijboer*³, *J. Klijn*³, *S.E. Plon*¹. 1) Baylor College of Medicine, Houston, TX; 2) Leiden University Medical Center, Netherlands; 3) Erasmus University Medical Center, Rotterdam, Netherlands.

Germline mutations in the *BRCA1* and *BRCA2* genes have been extensively studied in Caucasian populations including the Ashkenazi Jewish population. However, less is known about mutations in these genes in other population groups. In addition, most deleterious mutations interrupt the coding portion of the gene or the flanking 3' and 5' splice sites. We would like to report 2 new mutations at the same intronic residue, which have been identified in very highly affected African American and Dutch/Belgian families.

Family 1: The proband was a 44 years old African American woman diagnosed with breast cancer at age 27. Her family history was remarkable for 4 sisters with early-onset breast cancer with the age of diagnosis ranging from age 24-32 years old and a mother who had died of breast cancer at age 35. Her maternal grandmother had postmenopausal breast cancer. There were no cases of ovarian cancer. Her brother had colon cancer diagnosed at age 35. Full sequence analysis of *BRCA1* and *BRCA2* at the Myriad Genetic Laboratories Inc., revealed an intronic mutation in the *BRCA1* gene: IVS22+5G>T. She also had a known variant I3412V in the carboxy terminus of *BRCA2*.

Family 2: A Dutch/Belgian family highly affected with breast and ovarian cancer was studied. The proband was diagnosed at age 41 with breast cancer. Her family history included: 3 first degree relatives with early-onset breast cancer and 10 other relatives with early-onset breast and ovarian cancer. The age of diagnosis for breast cancer was from 30-45 years old. Analysis at the University of Leiden revealed the proband and a cousin carried a IVS22+5G>A mutation in the *BRCA1* gene which segregated with cancer in the family. cDNA analysis confirmed deletion of exon 22 which results in truncation of the protein.

This data suggests that mutation at IVS22+5 disrupts splicing at exon 22 in *BRCA1* and results in a high risk of very early-onset breast and ovarian cancer.

Analysis of MLL septin-like fusion (*MSF*) in breast cancers as a candidate 17q25 tumor suppressor gene. *L.M. Kalikin, C.K. Neeley, E.M. Petty.* Dept Internal Medicine, Univ Michigan, Ann Arbor, MI 48109-0638.

We cloned a novel 17q25 septin-like gene from a 300 kb breast tumor suppressor gene candidate interval that we defined by allelic imbalance studies. Interestingly, this gene was also identified as a fusion partner with *MLL* on 11q23 in acute myeloid leukemia patients and was named MLL septin-like fusion (*MSF*). Septins belong to a highly conserved GTPase subfamily, bind to membrane phospholipids, polymerize into filaments, and coordinate cell cycle progression. Yeast septins localize to the cleavage furrow and, when mutated, result in elongated buds, delayed mitosis, and multinucleated cells. Mammalian septins localize to the contractile ring with mutations leading to multinucleated cells. We have identified 3 alternatively spliced 5' exons that predict 3 amino-terminal distinct *MSF* protein isoforms. Other results predict two additional carboxy-terminal distinct isoforms. RNA expression studies using a probe common to all isoforms show differential expression of 3.0 kb and 4.0 kb *MSF* transcripts in all fetal and adult tissues tested.

Given that septins appear to play an integral role in the cell cycle, including cell division, signal transduction and cytoskeletal scaffolding, we hypothesize that altered *MSF* expression could contribute to carcinogenesis. Therefore we further investigated *MSF* as a positional candidate for the 17q25 suppressor gene. Northern blot analyses of breast tumor cell lines revealed 8 of 22 lanes with variable sized transcript bands not found in 5 normal breast cell lines. However, only silent polymorphisms were identified in the coding region of the 8 lines. Similarly no functionally significant nucleotide alterations were found in the coding region of 7 paraffin embedded breast tumors with minimal 17q25 allelic imbalance. These initial results do not support a direct role for *MSF* mutations in breast tumorigenesis. However, a role for alternatively spliced transcript expression has not been excluded. Further *MSF* expression and functional analyses are underway as well as characterization of other novel transcripts in the interval.

Loss of heterozygosity and RB1 mutations associated with retinoblastoma in Mexican patients. *L. Orozco*^{1,2,3}, *A. Atkinson*², *M. Macias*¹, *F. Garcia*^{1,3}, *C. Ridaura*¹, *M. Dean*². 1) Human Genetics and Pathology Departments, Instituto Nacional de Pediatría. Mexico, DF., Mexico; 2) Laboratory of Genomic Diversity, National Cancer Institute-FCRDC, Frederick, MD; 3) Interinstitutional Program of Molecular Biomedicine. CICATA-IPN. Mexico City, Mexico.

Retinoblastoma (RB) results from mutations or inactivation of both alleles of the RB1 gene. Approximately 40% of all cases are hereditary. Children with heritable RB are usually diagnosed within the first year of age, and develop bilateral or multiple tumors. Fifteen percent of unilateral RB cases are heritable. Children with nonhereditary RB tend to develop unilateral tumors at a later age than do children with the hereditary form of the disease. In Mexico, RB is the third most frequent cause of cancer in children and 56% develop the tumor during the first year of age. Although RB is usually recognized clinically, some forms of the disease can elude diagnosis. Here we report the results of a mutational analysis of blood samples and paraffin embedded RB tumors from 34 unrelated Mexican patients. Using SSCP and sequencing we identified 11 different RB1 mutations (33%), six of them have not been previously reported by the RB1-gene mutation database. We also characterized three novel polymorphisms. In two unilateral cases a RB1 mutation also was detected in peripheral blood DNA. Somatic mosaicism was suggested in one bilateral case without a detectable mutation in peripheral blood. A mutation was found in a mother without RB, revealing a non-penetrance case. Screening for BamHI and XbaI intragenic polymorphisms is underway. Our initial results show that loss of heterozygosity (LOH) at both loci might be less frequent among tumors where mutations were not detected. Our results suggest that the detection of RB1 mutations and LOH in Mexican population is lower than that observed in other populations mainly from developed countries.

BRCA1 mutation analysis in Greek breast cancer families. *A.C. Schofield¹, L. Ioannidou-Mouzaka², N.E. Haites¹*. 1) Medical Genetics, Univ Aberdeen, Aberdeen, Scotland; 2) Univ Athens, Greece.

Inherited predisposition to breast and ovarian cancer, which accounts for about 5-10% of these cancers, has been associated with mutations in the BRCA1 and BRCA2 genes. At present, the prevalence of BRCA1 mutations in Greek breast cancer families is unknown. We have identified 25 breast cancer families in the Greek population for BRCA1 mutation analysis. Of these, 6 families contain 1 breast cancer only (diagnosed under 35 years), 13 families with 2 breast cancer cases, 4 families with 3 breast cancer cases and 2 families with breast and ovarian cancer. The heterozygote risk of carrying an inherited predisposition breast cancer gene ranges from 6.7%-93.5%. The BRCA1 gene was screened for mutations by SSCP analysis. SSCP variants were further characterised by direct DNA sequencing. Our results to date have identified the presence of 5382insC mutation in 3/24 families (12.5%). In addition, a novel missense mutation, Pro1856Ser, has been identified in one case. Furthermore, a number of different sequence variants have been found in intronic and coding regions. Most of these represent polymorphisms, and studies are underway to determine the frequency of these variants in the Greek population. Our initial results suggest that the 5382insC mutation may represent a common founder mutation in the Greek population.

Characterization of functional mutations in the human estrogen receptor gene in breast cancer. *S.G. Roth¹, S.M. Gollin^{1,2}, M.D. Nichols^{2,3}*. 1) Department of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; 2) University of Pittsburgh Cancer Institute, Pittsburgh, PA; 3) Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA.

The human estrogen receptor (ER) acts as a transcription factor for estrogen-responsive genes in reproductive organs. The normal ER responds when bound by a ligand, which may either repress or activate genes depending on the conformation of the ER protein. The presence or absence of the ER in human breast cancer is utilized widely as a laboratory indicator for treatment approaches. Studies have suggested that tumors expressing the ER respond to tamoxifen, an anti-hormone drug. An abnormal conformational change results when the ER is bound by tamoxifen, thereby hindering the transcriptional activity of ER protein on estrogen-responsive genes. In certain ER-positive breast tumors, the response to tamoxifen does not repress transcription, instead activates it. In these tumors, perhaps a mutation in the gene coding for the ER results in abnormal protein function. Identifying mutations will help in understanding the role of an altered ER protein in breast cancer. A number of assays were used in this study to determine the genetic basis of ER dysfunction. Human breast tumors at various stages of disease were collected to recover RNA for ER cloning. A qualitative analysis examined how recovered human ER from tumors responds to a series of hormones and antihormones, using a fusion protein of the ER with a site-specific recombinant enzyme, FLP. Activation of the FLP-ER fusion protein complex led to excision of genetic markers. The assay indicated the presence of ER mutations through color changes after the FLP-ER complex was activated. Direct sequence analysis characterized mutations in altered ER clones, which revealed deletions and base pair mutations within the ligand binding domain of the ER that changed the amino acid sequence. These results confirm that certain deletion and point mutations in the gene coding for the ER in human breast tumors alter the conformational properties and subsequent activities of the ER protein.

Comprehensive mutational analysis of the ATM gene by denaturing HPLC. *Y.R. Thorstenson¹, P.J. Oefner¹, V. Goss², R.W. Davis¹, R.A. Gatti³, G. Chu².* 1) Stanford Genome Tech Ctr, Palo Alto, CA; 2) Stanford Univ Medical Ctr, Stanford, CA; 3) UCLA Medical Ctr, Los Angeles, CA.

ATM is the gene mutated in the autosomal recessive disease Ataxia-telangiectasia (A-T). ATM is a complex gene with 66 exons and 9168 base pairs of coding sequence in a genomic region of 150,000 base pairs. A worldwide effort to identify all of the mutations responsible for A-T has been hindered by the large size of the gene, as well as the heterogeneity of the causative mutations. Furthermore, most A-T patients are compound heterozygotes - a challenging situation even for conventional dideoxy sequencing protocols. Given these difficulties, it may not be surprising that published reports indicated a detection rate of only about 75%. Here, a new mutation detection strategy using denaturing HPLC (DHPLC), is compared with conventional protein truncation test (PTT), and single-strand conformation polymorphism analysis (SSCP). A set of 60 genomic DNA samples from 55 A-T patients and 5 obligate carriers was blinded to the DHPLC operator. The samples included mutations that had proven difficult to detect; only 69 out of 115 expected alleles had been identified previously (60%). DHPLC analysis confirmed all 69 and added 27 more alleles, for a total of 96 alleles (83%). Given that the sensitivity of DHPLC is close to 100%, it is likely that the remaining 19 alleles involve intron or flanking sequences in the ATM gene. The 96 alleles included 65 unique disease-causing mutations, including 23 not reported before. Of 19 unique mutations that were detected only by DHPLC, one was a single base pair insertion, and 18 were single nucleotide changes, clearly demonstrating a bias for detection of insertion and deletion mutations using conventional methods. Furthermore, the 18 newly detected single base pair changes were functionally relevant, including five premature stops, six non-conservative amino acid changes at residues conserved in the mouse ATM homolog, three alterations at canonical splice donor or acceptor sites, and four disruptions of potential splice enhancer motifs. Hence, many more of the previously undetected mutations in A-T patients may be caused by single base pair changes.

Sequence variation of the ARA24 gene and prostate cancer in African Americans. *R.K. Panguluri¹, W. Chen¹, C. Ahaghotu^{2,3}, W. Isaacs⁴, G.M. Dunston^{1,3}, R.A. Kittles^{1,2,3}*. 1) National Human Genome Center at Howard University; 2) Division of Urology, Howard University Hospital; 3) Howard University Cancer Center, Washington, DC; 4) Johns Hopkins Medical Institutions, Baltimore, MD.

ARA24 is an androgen receptor associated protein that has been shown to interact with the androgen receptor's (AR) N-terminal polyglutamine tract and enhances coactivation of the AR. In Kennedy's disease, ARA24 interacts weakly with longer AR polyglutamine tracts producing a weaker coactivation of the AR. Shorter polyglutamine tracts in the AR are more common among populations of African descent than other ethnic groups. The prevalence of prostate cancer is higher in African Americans compared to any other ethnic group and this may be attributed to the shorter AR polyglutamine repeats. Genetic variation of the ARA24 gene may increase or decrease coactivation of the AR leading to higher or lower activity of the AR. The purpose of this study was to screen the ARA24 gene for single nucleotide polymorphisms (SNPs) that may contribute to greater coactivation of ARA24 with smaller polyglutamine tracts of the AR in African Americans. African American prostate cancer cases and matched controls were screened for ARA24 SNPs by DHPLC and direct sequencing. The ARA24 gene has a single open reading frame of 651 bases that encodes a protein of 216 amino acids. We observed that the ARA24 gene sequence data in published databases such as NCBI contained many discrepancies in the sequence. A consensus sequence of the ARA24 gene was obtained by using African American, European, and Asian samples. Screening of the ARA24 gene revealed 5 non-synonymous mutations (T25P; V27M; E105D; L163W; S181A) and 1 synonymous mutation (G103G). One of these variants V27M was genotyped in prostate cancer cases (n=97) and controls (n=91). The frequencies of this allele are 0.07 and 0.06 in cases and controls, respectively. No association between prostate cancer and this variant was observed (p=0.8). The discovery of these novel SNPs will provide the opportunity to test for haplotype associations as well as genotype-genotype interactions with other androgen related genes influential in the etiology of prostate cancer.

A tumor suppressor gene for the development of cervical cancer has been localized to a region of 400 kb that contains 8 potential transcripts. *D.S. Gerhard¹, T.L. Nguyen¹, Y. Li², P. Huettner³, A. Mungall⁴, J.S. Rader².* 1) Dept Genetics, 8232, Washington Univ Sch Medicine, St Louis, MO; 2) Dept. of Obstetrics & Gynecology, Washington University SOM, St. Louis; 3) Dept. of Pathology, Washington University SOM, St. Louis; 4) 4Sanger Centre, Hinxton, England.

There are ~400,000 new cases of invasive cervical cancer (ICC) each year. A number of factors increase the risk of tumor development, but the most consistent risk factor is human papillomavirus (HPV) infection. HPV infection alone is not sufficient for the development of ICC since most infected women do not have tumors. We have previously determined that a large number of cervical tumors have lost all or part of 6p, suggesting that a TSG is localized there. We have also found deletions of 6p in the precursor lesion. About 50% of 139 invasive tumors, of all stages and histologic types, lost at least 6p21-6p23; some deletions were larger. A number of tumors have overlapping interstitial deletions of 6p23 and the composite information indicates that the boundary of the candidate region is defined by the markers D6S429 and D6S1578. This region of ~1 cM or ~1.4 Mb is covered by a contig of BACs and PACs. The contig allowed us to order 3 known, but unlocalized polymorphisms and from the recently generated sequence we developed 2 additional polymorphic loci. With these new markers we were able to identify one tumor whose deletion appears to be about 400 kb. Homology search of the 400 kb genomic sequence against the dbEST revealed the presence of at least 11 distinct clones. However, a careful examination of the EST versus genomic sequence revealed that at least 3 of the clones are genomic contaminants. We sequenced both 5 and 3 ends from the remaining 8 clones and found that 4 has an IVS, while the rest do not. One clone has only 600 bp, but does not have an orf. It is present in the cervix by RT-PCR and in situ hybridization. The expression of this gene in tumors is under investigation. Preliminary data show it to be overexpressed in ovarian carcinoma. Experiments are ongoing to determine it's function in cervical cancer. Experiments are also underway to investigate the other transcripts mapped to the candidate region.

Biochemical Characterization of the Mismatch Repair Protein MED1 (MBD4). A. Bellacosa^{1,2}, F. Petronzelli^{1,2}, A. Riccio¹, G.D. Markham¹, S.H. Seeholzer¹, J. Stoerker³, M. Genuardi², A.T. Yeung¹, Y. Matsumoto¹. 1) Human Genetics Program, Fox Chase Cancer Center, Philadelphia, PA; 2) Department of Medical Genetics, Catholic University Medical School, Rome, Italy; 3) Bruker Daltonics, Billerica, MA.

We recently isolated the human protein MED1 (also known as MBD4) in a two-hybrid screening using the mismatch repair protein MLH1 as a bait, and reported that MED1 has homology to bacterial base excision repair DNA N-glycosylases/lyases (Bellacosa, A. et al. *Proc. Natl. Acad. Sci. U.S.A.*, 1999). Via its 5-methylcytosine binding domain (MBD), MED1 binds to methylated DNA; this suggests that DNA methylation may affect MED1 repair processes. The MED1 (MBD4) gene is frequently mutated in human carcinomas exhibiting microsatellite instability (Riccio et al. *Nature Genet.*, 1999; Bader et al. *Oncogene*, 1999). In colorectal cancer specimens, we also detected loss of heterozygosity at the MED1 (MBD4) locus, suggesting that this gene may act as a tumor suppressor. Here we show that MED1 functions as a mismatch-specific DNA N-glycosylase active on thymine, uracil, 5-fluorouracil and, weakly, on the mutagenic adduct 3,N4-ethenocytosine, when these bases are opposite to guanine. The glycosylase activity of MED1 prefers substrates that contain a G:T mismatch within a methylated or unmethylated CpG site. Since G:T mismatches can originate via deamination of 5-methylcytosine to thymine, these findings indicate that MED1 may act as a caretaker of genomic fidelity at CpG sites. In order to gain insight in the molecular mechanisms of action of MED1, we conducted a kinetic analysis of the glycosylase reaction. This analysis revealed that MED1 displays a fast first cleavage reaction followed by slower subsequent reactions, resulting in biphasic time course. This is due to the tight binding of MED1 to the AP site reaction product rather than a consequence of enzyme inactivation. Comparison of kinetic profiles revealed that the MBD and methylation of the mismatched CpG site are not required for efficient catalysis, indicating that the roles of cytosine methylation and of the MBD in DNA repair by MED1 are independent of glycosylase activity.

A conserved gene antisense to the proto-oncogene *c-RAF* encodes a multi-zinc-finger protein, MAKORIN2. T.A. Gray¹, K. Azama², K. Whitmore¹, A. Min¹, S. Abe², R.D. Nicholls¹. 1) Dept Genetics, Case Western Reserve Univ, Cleveland, OH; 2) Faculty of Agriculture, Ehime Univ., Matsuyama, Japan.

High levels of *c-RAF* proto-oncogene expression occur in some human lung cancers, and recent studies have shown that over-expression of wildtype *c-Raf* in a mouse model leads to an increased incidence of lung tumorigenesis. The *c-RAF* proto-oncogene can be downregulated by antisense oligonucleotide therapy, culminating in a reduced growth rate for treated cells. Combined, the data suggest that regulation of *c-RAF* expression is an important factor in some cancers. Here, we describe a novel gene, MAKORIN RING zinc-finger 2 (*MKRN2*), that overlaps and is antisense to *c-RAF*. Northern blot analyses show that human and mouse *MKRN2* and *c-RAF* are concurrently transcribed, suggesting that the mRNA products may form RNA duplexes. We have also identified *MKRN2* orthologs in fish and birds, placing the gene duplication event that gave rise to this locus early in vertebrate evolution, at least 450 million years ago. All MAKORIN2 orthologs are well-conserved (56%-93% amino acid identity) and retain the hallmark RING and C3H zinc-finger composition of the MAKORIN protein family. While the function of MAKORIN2 is unknown, RING and C3H zinc-fingers suggest macromolecular and ribonucleoprotein roles, respectively, and poxviral derivatives homologous to the MAKORIN protein family circumvent apoptotic pathways. Taken together, our data identify a conserved vertebrate *MKRN2* gene that overlaps the *c-RAF* locus in a tail-to-tail orientation. The conservation of this arrangement implies that it is evolutionarily advantageous, possibly due to co-regulation of the *MKRN2* and *c-RAF* antisense mRNA and/or protein products. Present studies are examining a functional link between *MKRN2* and *c-RAF*, and evaluating *MKRN2* for disruption in human cancers.

An expression microarray for investigation of genes involved in loss of heterozygosity on chromosome 16 in breast cancer. *A.M. Cleton-Jansen^{1,2}, T. Van Wezel¹, E. Hogenhout¹, H.M. Van Beerendonk¹, M. Van den Berg², J. Den Dunnen², G.J.B. Van Ommen², C.J. Cornelisse¹.* 1) Pathology, LUMC, Leiden, The Netherlands; 2) Human and Clinical Genetics, LUMC, Leiden, The Netherlands.

Molecular-genetic as well cytogenetic studies have revealed that loss of heterozygosity (LOH) at the long arm of chromosome 16 (16q) belongs to the most frequent genetic alterations occurring in breast cancer. In a previous study we identified two separate smallest regions of overlap (SRO) involved in LOH at 16q22.1 and 16q24.3. A detailed investigation of genes in the SRO at 16q24.3 has so far not led to the identification of the gene targeted by LOH. We now want to use a different approach that will lead to the identification of the tumour suppressor gene(s) thereby making use of the wealth of data generated by the human genome project and the novel technique of cDNA expression arrays. Microarray construction and scanning is performed at the Leiden Genome Technology Centre. We are constructing a microarray containing partial cDNA clones of genes and ESTs (expressed sequence tags) that are mapping to the region which is most frequently involved in LOH, i.e. 16q22.1-qter. Information on the presence of transcribed sequences at this locus is obtained from the public databases such as Genemap '99, and from the large scale sequencing efforts. Special effort is put into obtaining the genes from the two SROs at 16q22.1 and 16q24.3. Genes and ESTs are PCR-amplified from a 40,000 clone library constructed by the I.M.A.G.E. consortium and PCR products are spotted onto microscope glass slides in an arrayed fashion. The microarrays are hybridized with labeled RNA from normal mammary epithelium versus breast cancer cells obtained from cultured cells or primary tumours. The result shows which genes are expressed in mammary epithelial cells, both normal and tumour cells. Furthermore it is anticipated that in a subset of the tumours a gene shows repeatedly decreased transcription. This microarray analysis will facilitate the selection of potential candidate genes, which will be further investigated in tumours showing LOH at chromosome 16.

A microcell hybrid based approach to identify chromosome 3p genes that antagonize tumor growth. I.D. Kholodnyuk, A. Szeles, M. Kost-Alimova, V. Kashuba, Y. Yang, G. Klein, S. Imreh. MTC, Karolinska Institute, Stockholm, Sweden.

We have previously shown that inoculation of human chr3/A9 mouse fibrosarcoma microcell hybrids (MCHs) into SCID mice was followed by the regular elimination of some 3p regions (Imreh et al., 1994). Using this approach, referred to as the elimination test (Et), we have defined a common eliminated region (CER) at 3p21.3 and an eliminated region (ER) at 3p21.1-p14.2 (Kholodnyuk et al., 1997). ER partially overlaps the FHIT gene, considered as a putative TSG. In the present work, the new MCHs and 13 derived SCID tumors, were analyzed by FISH and by PCR using 83 chr3-specific markers. Nine tumors generated from 3 MCHs remained PCR-positive for all chr3 markers tested ("PCR+" tumors). FISH showed normal intact chr3 in 65-98 % of MCHs cells and in 16-75% of tumor cells. Although FISH reverse painting (FISH-RP) has indicated losses over 3p26-3p25, 3p24, 3p22, 3p21 and 3p14. The "PCR+" tumors were examined by RT-PCR for the expression of 23 human genes: 5 genes within CER1 (LIMD1, CCR1, CCR2, CCR3, CCR5), 7 genes located within regions that were homozygously deleted in a variety of carcinomas (ITGA4L, SEMA4, GNAI2, LUCA1, PTPRG, FHIT, DUTT1), and 11 other genes in chr3p. We have found that VHL, MLH1, ITGA4L, NKTR, CTNNB1, LIMD1, UNPH, TCTA, APEH, UBE1L, SEMA4, LUCA1, PTPRG and DUTT1 were expressed in the SCID tumors as well as in the MCH lines in vitro. FHIT was expressed in vitro in 5 out of 7 MCH lines. Nine of 13 derived tumors had no FHIT transcript. The remaining 4 expressed a truncated mRNA. We have previously found that FHIT was deleted at the DNA level in 21 tumors derived from 4 MCHs. Our compiled data show that FHIT was either physically or functionally impaired in all 34/34 analyzed tumors. This suggests that the expression of the human FHIT gene in mouse fibrosarcoma cells impairs the growth potential of the cells in SCID mice. Interestingly that the transcript of the murine homologue of FHIT was lost also from the MCHs after growth in SCID mice. Comparative duplex RT-PCR revealed also significant reduction of the amount of GNAI2 transcript and DLEC1 transcript in SCID tumors versus the MCH lines in vitro.

Identification of Novel Tumor-Associated Genes in 11q12-q23. *R.A. Kuner, E. Dahl, C. Pilarsky, T. Specht, A.O. Schmitt, B. Hinzmann, B. Weber, M. Choschzick, E. Staub, A. Buss, B. Petschke, A. Rosenthal.* metaGen GmbH, Berlin, Germany.

Tumor development is characterized by chromosomal disorders which may lead to activation of oncogenes or loss of tumor suppressor genes. In gynecological tumors, genetic information within chromosomal region 11q12-q23 is often amplified or lost. Assuming that chromosomal rearrangements in tumors cause changes in gene expression, four million ESTs available in public and proprietary EST databases were subjected to in silico expression analysis using assembled cDNA sequences (Schmitt et al., 1999). In this first approach 600 candidate genes were identified which are associated with sporadic forms of gynecological tumors (Dahl et al., in preparation). Chromosomal assignment of these 600 genes localized 15 genes within 11q12-q23. To achieve an even higher coverage of tumor-associated genes for this critical chromosomal region we analyzed 968 STS-markers between RH13699 (11q12) and RH27416 (11q23) for expression analysis as aforementioned. Using this strategy we identified a total of 69 genes which are significantly differentially expressed in gynecological tumors. 47 of these genes have been described previously, whereas 22 are novel. Verifying these in silico data by Northern blot technique, RNA in situ hybridization and RT-PCR, we identified one novel gene in 11q14.1 which may represent a tumor suppressor gene.

Suppression of prostate cancer tumorigenicity by chromosome 18. *T.L. Johnson-Pais¹, S.S. Padalecki², K. Weldon³, A.M. Killary⁴, R.J. Leach^{1,3}.* 1) Dept Pediatrics, Univ Texas Health Sci Ctr, San Antonio, TX; 2) Dept Medicine, Univ Texas Health Sci Ctr, San Antonio, TX; 3) Dept Cellular & Structural Biology, Univ Texas Health Sci Ctr, San Antonio, TX; 4) Div of Laboratory Medicine, Univ Texas M.D. Anderson Cancer Ctr, Houston, TX.

Our recent studies of 32 metastatic prostate tumor samples have identified two non-overlapping regions of allelic imbalance on chromosome 18 that are associated with the progression of prostate cancer. We have also analyzed DNA obtained from the human prostate cancer cell lines DU-145 and TSU-PR1 for significant stretches of homozygosity using highly polymorphic markers from chromosome 18. Both of these lines exhibit an apparent loss of heterozygosity on the long arm of chromosome 18. DU-145 appears to have lost both regions, while TSU-PR1 appears to have lost only the more distal region at 18q22-q23. In an effort to identify putative tumor suppressor genes located within these regions on chromosome 18 that are involved in prostate cancer, we have used microcell-mediated chromosome transfer to introduce an intact chromosome 18 and chromosome 8 into TSU-PR1 cells. We have also introduced an intact chromosome 18 into DU-145 cells. While introduction of an intact chromosome 8 had no effect on the in vitro growth properties or the in vivo tumorigenicity of TSU-PR1 cells, microcell hybrids containing an introduced chromosome 18 exhibited a longer population doubling time, retarded growth in soft agar and slowed tumor growth in vivo. Introduction of chromosome 18 into DU-145 cells also had a profound effect on the in vitro growth of the hybrids, which precluded isolation of hybrid cells for further analysis. These experiments provide functional evidence for the presence of at least one tumor suppressor gene on human chromosome 18 involved in prostate cancer.

The *RET* proto-oncogene and its ligand *GDNF* are expressed in human head and neck tumors. *M.A. Nagai*¹, *L.P. Kowalski*², *L.M. Mulligan*³. 1) Disciplina de Oncologia, Depto de Radiologia, Fac de Medicina da USP, Sao Paulo, Brazil; 2) Fundacao Antonio Prudente, Sao Paulo; 3) Department of Pathology, Queen's University, Kingston Canada.

Germline mutations in the *RET* proto-oncogene are associated with multiple endocrine neoplasia (MEN) type 2 syndromes and Hirschsprung disease. In addition to that, somatic mutations in the *RET* gene have been demonstrated in human thyroid carcinomas. To date, there have been no reports describing the involvement of this proto-oncogene in human tumors from other anatomic sites of the head and neck. In the present study, using RT-PCR, we have examined the expression of *RET* proto-oncogene and *GDNF* transcripts in RNA samples from both tumor and normal tissue from 15 patients with head and neck carcinomas (oral cavity, 14 cases; larynx, 1 case). We have consistently detected *RET* and *GDNF* expression in the samples analyzed. Moreover, 4 out of the 15 tumors analyzed showed increased levels of *RET* transcripts. Further, using PCR-SSCP we have examined exons 10, 11, 13, 14, 15 and 16 of the *RET* proto-oncogene for the presence of mutations in these tumours which could be associated with the disease phenotype. These exons represent the sites of mutations previously associated with tumorigenesis. So far polymorphisms have been observed in exons 11, 13 and 15 but we have found no evidence of disease associated mutations. Here we show for the first time that transcripts of the *RET* proto-oncogene and its ligand *GDNF* are expressed in head and neck cancer. Further studies will be needed to clarify the role of the *RET* proto-oncogene in the initiation or progression of tumorigenesis in head and neck tumors. Supported by: PADCT/CNPq.

GFRA-3 variants in human lung carcinoma. C.I. Onochie¹, Y. Shi¹, J.B. VanHorne¹, R.M.W. Hofstra², L.M.

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GDNF family receptor a-3 (GFRA-3) is a co-receptor required for the activation of the RET receptor tyrosine kinase by its circulating ligand artemin. GFRA-3 is most highly expressed in the peripheral nervous system, heart, lung and neuroendocrine cell types. Using RT-PCR, we have shown that GFRA-3 is expressed in a series of small cell (SCLC) and non-small cell (NSCLC) lung carcinoma cell lines. We have previously shown that RET is also transcribed in these lines, suggesting that autocrine or paracrine activation of this system might play a role in tumour progression. To investigate such a role, we performed SSCP analyses of the *GFRA-3* gene in a panel of SCLC and NSCLC tumours. We have not detected mutations or variants of the coding sequence of the *GFRA-3* gene in this population, however, we have identified polymorphisms in introns 4 (IVS4-30A>G) and 7 (IVS7+4insGG) of the gene. The frequency of the intron 4 variant was similar in our tumour lines and control population (7%). However, we found that the IVS7+4insGG insertion was significantly over-represented in a panel of 52 SCLC and NSCLC lines (6%) as compared to our controls population (0.6%; $p < 0.005$). The IVS7+4insGG variant lies within the consensus splice donor site downstream of exon 7, changing it from a GTAGGGCC to GTAGGGGG, and thus has the potential for disrupting splicing of the *GFRA-3* transcripts. We are currently investigating the effect of this variant on *GFRA-3* transcription. The *GFRA-3* gene has been mapped to 5q31, a region of frequent allelic loss in SCLC. Our data suggest that, either *GFRA-3* or a locus linked to it within this region, may have a role in lung tumour development.

Identification of expression changes of prognostic and therapeutic value in metastasizing medulloblastoma. T.

MacDonald¹, K.M. Brown^{1,2}, P.H. Cogen¹, Y.-W. Chen¹, B. Rood¹, R.J. Packer¹, D.A. Stephan^{1,3}. 1) Childrens National Medical Center, Washington, DC; 2) George Washington University Genetics, Washington, DC; 3) National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Medulloblastoma is a highly malignant cerebellar tumor of children and, less frequently, of adults, with a tendency to early recurrence and dissemination. Despite recent advances in patient survival, the quality of life for survivors is poor due to neurocognitive, neuroendocrine, and hearing deficits as a direct result of the whole brain and spine radiation required for prevention of metastatic disease. Using global expression profiling, we identified genes prognostic of tumor metastasis that may also serve as new therapeutic targets. An initial expression scan was done using a pooling approach, assuming that genetic heterogeneity would not be a significant confounding factor. We isolated total RNA from six metastasizing and six-non metastatic tumors, made biotinylated cRNA, and pooled samples from the two clinical groups in equimolar amounts. Gene expression profiles were generated using Affymetrix HuGeneFL Gene Chips (5600 genes). 266 genes showed differential expression greater than two-fold. The 58 genes expressed at least five-fold more or less between groups were selected for further analysis. 21/30 genes with *increased* expression in metastatic tumors have been implicated in promoting: invasion/metastasis (14), cell growth (4), and angiogenesis (3). 12/28 genes with *decreased* expression in metastatic tumors have been implicated in inhibiting: invasion/metastasis (5), cell differentiation (6), and cell growth (1). Expression of each gene is being characterized via immunostaining (45/58 have available antibodies) and qRT-PCR using 48 frozen and 130 paraffin embedded tumors, and correlated with clinical and pathological data. This study has identified historically valid candidate genes involved in metastatic medulloblastoma, and has shown that a pooling strategy can be utilized effectively to identify candidate genes without complex statistical analysis, as long as validation studies are part of the study design.

Molecular genetic and mutational analysis of CDKN2A (p14/p16) in 260 human neuroblastomas. *P.M. Thompson, J.M. Maris, M.D. Hogarty, G.M. Brodeur, P.S. White.* Division of Oncology, Children's Hospital of Philadelphia, Philadelphia, PA.

Neuroblastoma (NB) is a common pediatric tumor arising from post-ganglionic sympathetic neuroblasts. Loss of heterozygosity (LOH) for several specific genomic regions, including 1p36, is frequently observed in NB tumors and cell lines. A panel of 46 NB cell lines was used to search for homozygous deletions (HD) within 1p36 and at 21 known tumor suppressor gene loci. HD was detected only at CDKN2A, in 4 cell lines. The CDKN2A locus encodes two overlapping proteins, p16 and p14, which are involved in cell cycle regulation and are inactivated in many human malignancies. HD was also detected in corresponding tumor specimens, but not in constitutional DNA samples, in two of the three cell lines for which tumor tissue was available. All four exons encoding p16 and/or p14 were homozygously deleted in three cell lines, while the fourth cell line had HD of only 104 bp of CDKN2A exon 2. To determine the extent of CDKN2A deletions in primary NBs, LOH analysis was performed upon 214 NB tumor and constitutional DNA pairs using four 9p21 polymorphisms flanking CDKN2A. Allelic loss of all four loci was present in 20 tumors and LOH for only a subset of informative markers was evident in 10 additional tumors (14% 9p21 LOH). Analysis of the deletion breakpoints for the 10 tumors with partial LOH showed 2 non-overlapping SROs: one region mapping proximal to D9S171 and including CDKN2A, and a second, more distal 9p21 region between D9S259 and D9S169. Five of 46 NB cell lines (11%) were homozygous for 4/4 markers within 9p21, suggesting allelic deletion of the entire region in these cell lines. Sequencing of CDKN2A exon 2 (shared by p16 and p14) and exon 1 (unique to p14) in 30 primary tumors with LOH of CDKN2A found no mutations. LOH for 9p21 was correlated with 1p LOH ($p < 0.05$) and 14q LOH ($p < 0.01$) but not with MYCN amplification, tumor stage, patient age, histopathology, or 11q LOH. These results suggest that biallelic inactivation of CDKN2A may contribute to tumorigenicity in a subset of NBs, and that two or more tumor suppressor loci involved in NB tumorigenesis may be located in proximal 9p.

Adenoviral transfer of the FHIT gene in cancer cell lines results in apoptosis and activation of the caspase-8 pathway. *L. Roz¹, M. Gramegna¹, P. Accornero¹, H. Ishii², C.M. Croce², G. Sozzi¹.* 1) Dept Experimental Oncology, Ist Nazionale Tumori, Via Venezian 1, 20133, Milano, Italy; 2) Kimmel Cancer Center, Jefferson Medical College, Philadelphia, Pennsylvania 19107.

Although the inactivation of the FHIT tumor suppressor gene seems to play an important role in different types of human cancer, the mechanism of action responsible for the oncosuppressive properties of the protein is still unknown. In the present study we have used an adenoviral vector to express the Fhit protein in 7 lung and 3 cervical cancer cell lines with low or undetectable level of endogenous protein and analysed the effect on the cell cycle and on apoptosis as measured by TUNEL assay. High levels of expression of the transgene could be achieved in all the cell lines tested and the efficiency of transduction was also confirmed by experiments done with control adenoviral vectors expressing lacZ or GFP. The levels of apoptosis induced by the reintroduction of Fhit expression ranged from 15% to 55% depending on the cell line and on the culture conditions: serum deprivation seemed to increase the pro-apoptotic effect of Fhit. Both lung and cervical cancer cell lines were susceptible to Fhit mediated apoptosis with as low as 10% of cells remaining viable 5 days after gene transfer. Analysis of the cell cycle at different time points after transduction showed a progressive block in the cycling properties of the cells with accumulation in the S-phase before massive cell death. In vitro transduced H460 cells also lost their ability to induce tumor growth in vivo in nude mice. Finally, in Fhit transduced cells we could consistently detect activation of caspase-8, suggesting the involvement of this signalling pathway in Fhit induced apoptosis. In accordance with this observation treatment of H460 cells stably transfected with a Fhit expression plasmid with agonistic anti-Fas antibody resulted in high apoptotic rate confirming that cells expressing high levels of Fhit might be particularly susceptible to caspase-8 mediated apoptosis. In conclusion, our results show high apoptotic activity and anti-tumorigenic properties of the Fhit protein, possibly mediated by interaction with the caspase-8 pathway.

VNTR polymorphism of HRAS1 gene in breast cancer patients in Tomsk region of Russia. *I.V. Tereschenko¹, M.V. Goloubenko², V. Saviouk².* 1) Prevention Department, Cancer Research Institute, Tomsk, Russia; 2) Population Genetics Lab, Institute of Medical Genetics, Tomsk, Russia.

Recent studies failed to support an association of rare VNTR alleles of HRAS1 gene with susceptibility to neither hereditary nor early-onset breast cancer. However, numerous previous investigations with the use of PDRF analysis showed the link of that polymorphism with increased risk of breast cancer. To investigate whether the breast cancer risk is associated with rare HRAS1 alleles we conducted a population based research of VNTR polymorphism of this locus using the PCR-based technique. We have genotyped 73 women with breast cancer from Tomsk region, Russia. The control consisted of 181 healthy individuals from Tomsk region. Mean age of the individuals from the group with breast cancer was 45,6 (29-68) years, that of control - 47,6 (32-82). Genomic DNA was extracted from blood samples and amplified using the protocol of Phelan et al. (1996). Amplified products were resolved by electrophoresis on 2% agarose gel (2,5 v/cm, 10 hours). Gels were stained with ethidium bromide. The allele frequencies for a1, a2, a3, a4 were 0.071, 0.011, 0.006, 0.001 in case subjects and 0.079, 0.008, 0.004 and 0.001 in control. The frequency of the rare alleles was 0.009 and 0.006, respectively. That difference was not significant (chi-square=1.16, p=0.28). In multiple cancer patients with primary tumor in breast (n=9) the frequency of rare alleles was 0.017 (chi-square=2.86, p=0.09). In group of patients with two or more first-degree relatives affected with breast cancer (n=8) 0.019 (chi-square=3.68, p=0.05).

Mutator phenotype at *Aprt* and *Hprt* loci in normal tissues of mice deficient in *Pms2*. C. Shao¹, M. Yin², L. Deng¹, R.M. Liskay³, T.C. Doetschman², P.J. Stambrook², J.A. Tischfield¹. 1) Rutgers Univ, Piscataway, NJ; 2) Univ. of Cincinnati College of Medicine, Cincinnati, OH; 3) Oregon Health Sciences Univ, Portland, OR.

The DNA mismatch repair (MMR) gene *Pms2* has been implicated in hereditary non-polyposis colon cancer. *Pms2* knockout mice exhibit predisposition to lymphoma, microsatellite repeat instability, and failure of spermatogenesis. To study the effects of *Pms2* deficiency on genomic integrity in somatic cells *in vivo*, we analyzed point mutation and loss of heterozygosity (LOH) at *Aprt* locus in T lymphocytes and fibroblasts of *Pms2*^{-/-}*Aprt*^{+/-} mice. The frequency of DAP-resistant (DAP^r) T lymphocyte clones, due to point mutation or LOH of *Aprt*, was about three fold higher in *Pms2*^{-/-} mice than in wild-type mice. While the majority (80%) of the DAP^r clones were caused by mitotic recombination in wild-type mice, point mutation was nearly as common as mitotic recombination in mutant T cell clones from *Pms2*^{-/-} mice. Also, the frequency of 6-TG-resistant (6-TG^r) T cell clones, due to mutation of *Hprt*, was increased about sixty-fold in *Pms2*^{-/-} mice. The point mutations were predominantly caused by T:A to C:G transition. The frequency and mutational spectrum of DAP^r fibroblasts in *Pms2*^{-/-} mice was not significantly different from those in *Pms2*^{+/+}, though 6-TG^r fibroblasts were more common in *Pms2*^{-/-} mice. The mutator phenotype as a consequence of MMR deficiency may play a role in initiating and/or accelerating carcinogenesis.

Identification of differentially expressed genes in early and late stage primary ovarian tumors by the construction of suppression subtraction hybridization cDNA libraries. *D.I. Smith¹, A. Sen², R. Avula¹, J. Staub¹, J. Lee², L. Hartmann³, J. Lillie², V. Shridhar¹.* 1) Dept Experimental Pathology, Mayo Clinic & Foundation, Rochester, MN; 2) Millenium Predictive Medicine Cambridge, MA; 3) Division of Oncology, Mayo Clinic & Foundation, Rochester, MN.

Each year 16,000 American women succumb to ovarian cancer, the deadliest of gynecologic cancers. Ovarian cancer is frequently asymptomatic in its early stages, thus 75% of patients have advanced stage disease at the time of diagnosis. The anticipated 5-year survival for patients with advanced stage disease is less than 20%, while early stage disease is highly curable. In order to identify genes that are differentially expressed in early versus late stage ovarian cancer, we generated up and down suppression subtraction hybridization cDNA libraries from early and late stage primary ovarian tumors subtracted against normal ovarian epithelial cell brushings. Approximately 3000-4000 successful sequences were generated from each library. We analyzed the differentially expressed down regulated clones obtained from two individual early stage tumor libraries and compared them to clones from libraries generated from two individual late stage tumors. This analysis revealed that while there were specific genes that were unique to each library, the majority of the genes were common between the different libraries. Some of the genes that were down regulated in both early and late stage tumors included SPARC, osteoblast specific factor, caveolin 1, IGF2, glutathione transferase A2, HSP90, fibronectin, transferrin receptor, integral membrane protein 2A, and C7 complement factor. The differential expression of these genes were confirmed by semiquantitative RT-PCR with GAPDH as control in a panel of 15 stage I tumors and 25 stage III tumors of different histologies. Cadherin13, MMP2 and IL8 were only isolated from libraries generated from stage III/IV tumors and were not isolated from tumors generated from early stage tumors. We are in the process of characterizing novel clones that were isolated from these libraries to test them as potential candidate tumor suppressor genes that may be involved in the pathology of this malignant disease.

Post-translational modifications of OVCA1 contribute to ovarian carcinogenesis. *A.H. Prowse, W. Bruening, A.K. Godwin.* Fox Chase Cancer Ctr, Philadelphia, PA.

OVCA1 is a candidate tumor suppressor gene which maps to a region of frequent allelic loss in ovarian cancer at 17p13.3. *OVCA1* is mutated in some ovarian tumor cell lines and its protein levels are decreased or lost in nearly 40% of ovarian adenocarcinomas. Expression of low levels of exogenous OVCA1 results in dramatic growth suppression and decreased levels of cyclin D1 (Bruening *et al* 1999). *OVCA1* codes for a highly conserved protein with no known function, but the C-terminal region of OVCA1 interacts with an RNA binding motif protein named RBM8A (Salicioni *et al* 2000). We have recently found that OVCA1 exists in at least two forms: a 48 kDa and a 50 kDa protein. Subcellular fractionation studies indicate that p50^{OVCA1} localizes to the cytoplasm whereas p48^{OVCA1} is predominantly found in the organelle fraction with some in the nuclear fraction. Immunohistochemical analysis of normal ovaries and ovarian tumors using p50^{OVCA1} or p48^{OVCA1} specific antibodies demonstrated strong nuclear and cytoplasmic staining in the epithelial cells of the normal ovaries and borderline tumors for both antibodies. In contrast, p50^{OVCA1} was observed primarily in the nucleus, with little or no cytoplasmic staining in the vast majority of malignant ovarian tumor cells. Interestingly, no nuclear or cytoplasmic staining was detected in these adenocarcinomas using antibodies for p48^{OVCA1}. Furthermore, there was not apparent correlation with OVCA1 expression and cellular proliferation (as determined by ki67 staining) and BRCA1 levels in these tumors. 2-D gel western blotting revealed that OVCA1 is extensively modified post-translationally in tumor cells leading to a dramatic shift in pI from ~9 to ~7. These aberrant modifications result in the p50^{OVCA1} form and loss of immunoreactive p48^{OVCA1}. Studies of the molecular mechanisms regulating OVCA1 expression and localization, and its interaction with RBM8A are underway. Our studies indicate that altered expression and/or post-translational modifications of OVCA1 is associated with the development of malignant ovarian tumors and suggest a potentially new mechanism for the inactivation of tumor suppressors in ovarian adenocarcinomas.

Identifying Downstream Events Associated with Thy-1 Expression. *J. Xu, H.R. Abeyasinghe, N. Wang.* Dept Pathology & Lab Med, Univ Rochester Medical Ctr, Rochester, NY.

Previous studies with transfection and xenographic analysis in our laboratory have demonstrated that Thy-1, a membrane glycoprotein, is a putative tumor suppressor gene for ovarian cancer. Transfection of Thy-1 into SKOV-3, a tumorigenic human ovarian carcinoma cell line, resulted in reductions in tumor growth compared to their null counterparts while abolishing Thy-1 expression from 11(c)9-8, a non-tumorigenic hybrid derived from SKOV-3, restored tumorigenicity. In this study we performed reciprocal cDNA subtractions between SKOV-3-Thy-1 and SKOV-3-Null to identify the downstream effects of the Thy-1 gene. The cDNA pool generated from the reciprocal cDNA subtractions were then subjected to gene discovery array screening and to PCR-based differential display (DD-PCR) using four arbitrary primers. The array analysis revealed that 15 ESTs plus 33 known genes were up-regulated and 42 ESTs plus 61 known genes were down-regulated in SKOV-3-Thy-1 vs. SKOV-3-Null. Various tumorigenicity related known genes were found affected by Thy-1. Among the up-regulated genes, acid ceramidase, which has been confirmed by DD-PCR as well, is known to be involved with the induction of apoptosis and has been characterized as an anti-oncogene. Of the down-regulated genes, Cyclin D1 has been identified by both DD-PCR and microarray screening amplification and over expression of Cyclin D1 has been identified in a variety of tumor cells including breast, colon, and small cell lung cancers. The data obtained from this study provides some insight into the downstream genes that may have been affected by Thy-1 in the tumorigenic ovarian carcinoma cells and the possible mechanisms of Thy-1 in ovarian tumor suppression.

Genes up- and down-regulated by Thy-1 in an ovarian cancer doxycyclin Thy-1 inducible system. *N. Wang¹, W. Kao², K. Peck², J. Xu¹, H.R. Abeyasinghe¹.* 1) Dept Pathology, Univ Rochester Sch Medicine, Rochester, NY; 2) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, ROC.

A Thy-1 inducible expression system was established by co-transfection of two plasmids pTEP4m and pUHD172-neo into human carcinoma cell line SKOV-3. Three hours post doxycyclin exposure, Thy-1 expression is detectable. To identify the downstream gene expression of Thy-1, mRNA was extracted from the cells at 0, 3, and 6 hrs post doxycyclin exposure. cDNA was generated from various time points and then subjected to a high-density cDNA microarray analysis. The genes which expressed more than a 2 fold increase from both 0 to 3 hrs as well as 3 to 6 hrs were classified as up-regulated genes. These include homosapions immunoglobulin heavy chanin variable region (VH4), alanine aminotransferase, adenosine deaminase, and B-cell CLL/lymphoma 7b, etc. The genes which expressed 2 fold decreased from both 0 to 3 hrs as well as 3 to 6 hrs were classifed as down-regulated genes. These include protein phosphatase type IVA, involucrin, protein phosphatase 1, catalytic subunit, gamma isoform, and transporter 2, ABC (ATP banding cassette). In addition, there are genes that fit the criteria between 0 to 3 hrs but not between 3 to 6 hrs which may indicate early events immediately post Thy-1 induction.

5qNCA, a candidate for the chromosome 5 leukemia tumor suppressor gene, is a novel nuclear receptor coactivator. *C.A. Westbrook¹, Z. Arbieva¹, J. Kravarusic¹, B. Chyna¹, Z. Hu¹, S. Edassery¹, S.K. Horrigan².* 1) Department of Medicine, University of Illinois at Chicago, Chicago, IL; 2) Department of Pediatrics, Georgetown University Medical Center, Washington, D.C.

Loss or interstitial deletion of the long arm of chromosome 5, del(5q) or 5q-, is a consistent finding in acute myeloid leukemia (AML) or myelodysplasia (MDS), especially those that arise after previous chemotherapy. We and others have proposed the presence of a tumor suppressor gene (TSG) within the consistently-deleted region, which centers at 5q31. We report a novel gene at 5q31, 5qNCA, as a candidate for this TSG. 5qNCA produces a 7.4-kb transcript with a 5,286-bp open reading frame that is ubiquitously expressed and is present in CD34+ cells and AML cell lines. The gene spans 23 exons and contains a signature motif for a nuclear receptor co-activator, a novel zinc finger motif, and a highly-conserved C-terminus with putative transcriptional regulatory activity. It is homologous to TRIP8, human/murine hairless, KIAA0742, and drosophila CG815 in the zinc finger and transactivation domains. The zinc finger has the unique spacing CysX2-Cys-X7-His-X2-Cys-X2-Cys-X4-Cys-X2-Cys, which may define a new family, resembling both LIM and PHD zinc finger domains, which are known to regulate chromatin structure and are often involved in leukemia. Sequencing of KG-1, an AML cell line with del(5q), shows an apparent mutation in exon 6 resulting in a THR to ALA substitution which is not seen in >40 normal alleles. KG-1 thus demonstrates the loss of one allele and mutation of the residual allele, as expected for a TSG. Additional sequencing is in progress in clinical del(5q) samples. We propose 5qNCA as a likely candidate for the del(5q) TSG based on its protein homologies, expression, and mutation.

***EPHX1, GSTM1, GSTT1 and GSTP1* gene polymorphisms in patients with mouth and throat neoplasias.** A.G. Amador^{1,2}, P.D. Righi², S. Radpour^{2,3}, E.T. Everett^{1,2}, E. Weisberger², M. Langer², G.J. Eckert², A.G. Christen¹, S. Campbell, Jr.^{1,3}, D-J. Summerlin¹, J.K. Hartsfield, Jr.^{1,2}. 1) Indiana University School of Dentistry, Indianapolis, IN; 2) Indiana University School of Medicine, Indianapolis, IN; 3) Roudebush Veterans Administration Medical Center, Indianapolis, IN.

Xenobiotic metabolizing enzymes have been implicated in the carcinogenesis of tobacco-related neoplasias. Since polymorphisms are known to occur in genes coding for some of these enzymes, a search to determine representation of these polymorphisms in patients with mouth or throat cancer compared to a population sample was undertaken. Subjects included 120 ever-smokers, 15 never-smokers and 99 random controls. Polymorphisms were determined in genomic DNA using PCR followed by RFLP analysis for *GSTP1* and *EPHX1*, and by allele specific multiplex PCR for *GSTM1* and *GSTT1*. At *EPHX1* codon 113, a significant over-representation of the reportedly greater *in vitro* specific activity phenotype (Tyr/Tyr) was observed when compared to controls. This was observed for male ever-smokers (P=0.012) and for all ever-smokers (P=0.001), as well as in never-smokers (P=0.006). A less dramatic but significant over-representation of the Tyr/Tyr phenotype was observed in female never-smokers (P=0.042). There was an over-representation of homozygosity for the *GSTT1* null allele (but not for the *GSTM1* null allele) in ever-smokers in general, when compared to controls (21.45% vs. 10.10% P=0.027). Analysis of polymorphisms at the *GSTP1* locus did not show differences versus controls. However never-smokers, with a history of mouth or throat cancer had a 3.5 times higher incidence of the low activity producing phenotype B/B compared to ever-smokers (P=0.043) with a history of cancer. The *EPHX1* codon 113-Tyr/Tyr variant as well as homozygosity for *GSTT1* null have a role in, and/or are possible predisposing markers for carcinogenesis in the mouth and/or throat. Supported by grants NIH HD0737 (AGA) and NIH R29DE11280 (JKH).

The evolution of breast and ovarian cancer gene research: a bibliometric study. *L. Bouchard¹, R. Dalpé^{1,2}, D. Ducharme^{1,3}.* 1) CIRST, Univ De Quebec Montreal, Montreal, Qubec, Canada; 2) Political Sciences Department, Univ Montreal, Montreal, PQ, Canada; 3) Sociology Department, Univ Montreal, Montreal, PQ, Canada.

The main objective of this paper is to describe, using a bibliometric approach, the nature of scientific production in the field of cancer genetics, specifically with respect to the localization and identification of the BRCA genes (breast and ovarian cancer genes). Method: The Data were extracted from the Science Citation Index (SCI), the Social Science Citation Index (SSCI) and the Arts and Humanities Citation Index (AHCI). The search strategy consisted in choosing all papers that included "BRCA" in their titles. About 800 papers were retained, running from 1993, when the label was first introduced, to 1998. The extracted information included citations, quotations and authors (by countries and institutions), but excluded patents. This method allowed us to define research profiles by identifying the organizations involved in the research, the topics of investigation and the dynamics of scientific development. Results: The results revealed, among other findings, a steady increase in the number of articles related to BRCA genes: from 45 articles in 1993-94 to over 500 in 1997-98. These articles were published in specialized journals in three research domains: human genetics, oncology and medicine. The database contained mainly biomedical articles (97%) and relatively very few articles dealing with the psychosocial aspects of genetic screening (3%). About 15 articles were cited more than 100 times and a dozen of which generated at least two commentary, which gives an indication of the controversy surrounding this research area. American researchers accounted for about half of the international BRCA genes research community and were followed by English, Canadian and French researchers. The largest organizations involved were academic research centers, although private firms, like Myriad Genetics in Salt Lake City, played a very important role. Conclusion: The results allow us to portray the evolution and constitution of research groups and the configuration of research practices in terms of two major models: the "Republic of Science" and the privatization of fundamental knowledge.

Genotypic distribution of glutathione-S-transferase (GSTM1) in healthy subjects and in lung cancer patients in mexican population. *B.C. Gomez¹, M.P. Gallegos¹, J.I. Martinez², G. Morgan², A.L. Zamora¹, O. Torres¹, G.M. Zuniga¹.* 1) Medicina Molecular, CIBO, IMSS, Guadalajara, Jalisco, Mexico; 2) Division de Oncologia, Hospital de especialidades, CMNO, IMSS, Guadalajara, Jalisco. Mexico.

The enzymes GSTM1 belong to the isoenzymes family who participate in the desactivation of toxic compounds. Previous studies correlate the deletion of the GSTM1 gene with the increase to develop cancer, specially lung and bladder cancer in different world populations. The objective of this study was, determine the genotypic distribution of GSTM1 in healthy subjects and lung cancer patients in mexican population. 120 controls were study (52 smokers, 38 nonsmokers and 30 passive smokers) and 40 lung cancer patients, in order to take a peripheral blood sample (5 ml) for the DNA extraction. Using PCR, a segment of 271 bp, part of the exon 4 in the GSTM1 gene, was amplified with the primer G1 and G2 who coamplified with a 93 pb fragment in the exon 10 of Cystic fibrosis used as a internal control. The results of the genotypic distribution of the patients with lung cancer was 63.75 % for the allele present (+), and the 36.25 % for the null genotype when were compared between groups; no smokers 62% 38%; passive smokers 53%, and 47%, and smokers 63%, and 37% of the present and null genotypes respectively. The not shown difference statistical significance, but in the passive smokers group a tendency of GSTM1 null genotype were observed, showing the harmful effect of tobacco in that group.

Cytochrome *P450-1B1* genetic polymorphism, alcohol consumption and breast cancer risk. A.C. Deitz¹, R. Sachdeva², J. Swoyer¹, S. Gunasegaram¹, T.R. Rebbeck¹. 1) Center for Clinical Epidemiology & Biostatistics, Univ. of Pennsylvania, Philadelphia, PA; 2) Dept. of Pathology & Laboratory Medicine, Univ. of Pennsylvania-VA Health Care System, Philadelphia, PA.

Breast cancer etiology is multifactorial and complex. Factors associated with breast cancer risk include genetic susceptibility and exposure to estrogens. Alcohol consumption is thought to influence estrogen levels, which in turn may affect breast cancer risk. CYP1B1, a member of the cytochrome P-450 family, is involved in the metabolism of estrogens. We hypothesized that alcohol exposure and inherited genotypes at CYP1B1 may act synergistically to influence endogenous estrogen metabolism, and therefore affect breast cancer risk. To evaluate whether CYP1B1 interacts with alcohol consumption in breast cancer etiology, we designed a case-case study of peri- and post-menopausal breast cancer cases (n=227), 50-80 years of age. CYP1B1 genotypes were determined by PCR-based methods and alcohol consumption information was collected using a standardized questionnaire. For women with at least one variant allele at codon 432 (Val@Leu) consuming moderate to high levels of wine and hard liquor, we found an interaction odds ratio (OR) of 2.16 (95% CI=1.09-4.32). In addition, we found an interaction OR of 3.18 (95% CI=1.32-7.65) for women with two variant alleles who consume moderate to high levels of wine and hard liquor. These results suggest that a gene-environment interaction exists between alcohol consumption and CYP1B1, and that factors affecting endogenous estrogen metabolism may influence post-menopausal breast cancer risk.

Association between polymorphisms of the *GPX1* gene and multiple primary tumours (MPT). *S. Jefferies*¹, *Z. Kote-Jarai*¹, *R. Houlston*¹, *M-J. Fraser-Williams*¹, *R. A'Hern*¹, *W.D. Foulkes*³, *D. Goldgar*⁴, *R.A. Eeles*^{1,2}. 1) Cancer. Genetics, Inst. of Cancer Research, London, England; 2) The Royal Marsden Hospital Trust, Downs Rd, Sutton, Surrey, SM2 5PT; 3) McGill University, Montreal, Canada; 4) IARC, Lyon, France.

Head and neck cancer is a common cancer. There is some preliminary evidence that there may be a genetic component to SCCHN. Individuals who have had an index SCCHN have a 10-20% chance of developing MPT. There is an increased risk of cancer in first- degree relatives of individuals with SCCHN and this risk rises markedly when the individual has MPT. Individual susceptibility to cancer development may be contributed to by differences in metabolism of carcinogens. The *GPX1* gene is a selenium-dependent enzyme that maps to chromosome 3p21, an area frequently showing loss of heterozygosity on SCCHN. There are three *GPX1* alleles characterized by the number of GCG triplet nucleotide repeats encoding for alanine in a polyalanine tract in exon 1. These alleles are identical except for the length of the polyalanine polymorphism. The *GPX1/ALA6* also has a leucine for proline substitution at codon 198, a T for C substitution at +2 and a G for A substitution at -592¹. The effect on function of the *GPX1* gene is not known. We investigated the association between polymorphisms in the *GPX1* gene and patients with index SCCHN and MPT compared with population controls. The genotypes were determined for 63 cases of MPT and 259 controls using a PCR technique with a fluorescent-labelled primer. The PCR products were analysed using an ABI automated fluorescent DNA sequencer. The associations between specific genotypes and the development of MPT were examined by the Chi-square test. We found a significantly increased frequency of the *GPX1/ALA6/GPX1/ALA7* genotype in the MPT cases versus controls (p=0.04). The findings of this study suggest that certain *GPX1* genotypes may be associated with an increased risk of developing MPT. Identification of such genotypic markers may be of value in the future for targeting chemoprevention.¹ Moscow *et al* Carcinogenesis; 15:2769-2773, 1994.

SNP Identification and Haplotype Studies at BRCA2. *S.H. Jones, D.L. Nelson.* Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX.

This project seeks to identify functional variants of genes involved in familial cancers. These genes may play a role in increased or decreased risk for cancer predisposition in the general population due to their roles in DNA replication, repair or general cell maintenance. We have carried out SNP identification for BRCA2 and its surrounding genomic sequence, and defined haplotypes using ten CEPH families as well as individuals representing the four major ethnic groups from the U.S. (Asians, Caucasians, African Americans, Hispanics). The genomic region spanning approximately 180 kb was contained in two clones with a 3kb gap in between. A total of 10 non-coding SNPs were found, and seven of those, along with two previously reported (*Wagner et al.*, 1999) were used to type 321 individuals and to define haplotypes. We used the software package EMHAPFRE based on a maximum likelihood algorithm to infer haplotypes. EMHAPFRE analysis on the nine SNPs revealed 83 haplotypes when all samples were considered together. Two of the SNPs were located in the second clone containing the last three exons of BRCA2, and these loci appeared to be in equilibrium with the more proximal markers. This was further supported by EMHAPFRE analysis on the remaining seven loci, which decreased the total number of haplotypes to 50. Recombination and linkage disequilibrium analyses suggest historical recombination events to have taken place between the clones. DNA recombination and linkage disequilibrium was assessed using DNASP. These findings are relevant to the selection of markers to be used for association studies based on SNPs.

The ATM variant D1853N in BRCA1 mutation carriers. *R.A Omaruddin¹, K.L Nathanson¹, T.R Rebbeck³, B.L Weber^{1,2}.* 1) Department of Medicine, Univ of Pennsylvania, Philadelphia, PA; 2) Department of Genetics, Univ of Pennsylvania, Philadelphia, PA; 3) Department of Biostatistics & Epidemiology, Univ of Pennsylvania, Philadelphia, PA.

Mutations in BRCA1 confer a greatly increased risk of breast cancer. However, the breast cancer risk due to mutations in BRCA1 varies from 40-85%, depending on the population ascertained. Therefore it has been hypothesized that the mutation alone does not explain the observed pattern of cancer, but that other factors, including variants in genes other than BRCA1 and BRCA2, influence the development of breast cancer in BRCA1 mutation carriers. As ATM phosphorylates BRCA1 and both proteins participate in regulation of the DNA damage response, variants in ATM are candidates to modify BRCA1 penetrance. In addition, various studies have demonstrated an increased risk of breast cancer in female ATM heterozygotes. We have preliminarily investigated the G→A variant at nucleotide 5557 (D1853N) in 150 BRCA1 mutation carriers using PCR-RFLP analysis. The date of birth of the BRCA1 mutation carriers ranged from 1899 to 1977; 96 had developed breast cancer and 25 had developed ovarian cancer. In the BRCA1 mutation carriers, we were unable to find a statistically significant association between breast cancer ($p=0.611$) or ovarian cancer ($p=0.571$) and the ATM D1853N variant. Despite the lack of evidence for an association of this polymorphism and breast or ovarian cancer risk, the present analysis may have had limited statistical power to detect an association, and further evaluation of the ATM D1853N variant in an expanded set of BRCA1 and BRCA2 mutation carriers may be required.

Improved PCR-Genescan Genotyping of Mononucleotide Repeat (BAT) Loci. *S. Rao, W. Bloch.* Dept Sci & Technology, PE Biosystems, Foster City, CA.

The A-repeat BAT loci are useful in studying microsatellite instability (MSI), which correlates with certain cancers. They also represent a ubiquitous class of genetic polymorphism, possibly more common than either microsatellites with longer repeat units or SNPs. PCR-sizing methods are limited in resolving closely spaced mononucleotide-repeat alleles by two PCR artifacts, stutter and the terminal transferase side reaction exhibited by non-proof-reading enzymes. In the MSI field, BAT loci have been described as pseudomonomorphic, indicating that their natural size polymorphism lies close to the resolution limit of PCR-sizing methods. The present research provides increased control of PCR artifacts to improve confidence in mononucleotide-repeat genotyping, specifically at loci with high heterozygosity. The data shown here confirm that BAT loci are naturally polymorphic and provide allele distributions for several of these loci in a single population. Improved analysis of BAT-locus polymorphism should increase discrimination of acquired MSI. In addition, loss of heterozygosity correlated with cancer should become easier to detect for BAT loci. Finally, improved PCR-Genescan resolution of mononucleotide repeats increases their value as mapping and identification tools when more commonly used loci are insufficiently informative.

Lack of association between *HER2*^{V655} and breast cancer in a US population. C. Keshava¹, E.C. McCanlies¹, M.S. Wolff², A. Weston¹. 1) National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, WV; 2) Mount Sinai Medical Center, New York, NY.

A recent study in a population of Chinese women revealed an association between inheritance of a moderately common polymorphism (0.10-0.15) of *HER2* (I655V) and excess risk of breast cancer (OR = 12.7, 95% CI = 1.6-99.5) (Xie *et al.*, *J. Natl. Cancer Inst.*, 92:412-417, 2000). Also, a stronger relationship was claimed in younger women (adjusted OR = 1.7, 95% CI = 1.1-2.6). We tested this hypothesis in a population of US women enrolled in a hospital based case control study in New York City (n = 137 cases and 291 controls, matched 1:2 on age and self-reported race/ethnicity). No evidence was found to support an association between inheritance of the 655-valine variant of *HER2* and excess breast cancer risk. Our population consisted of African-Americans (n = 34 cases/58 controls), Caucasians (n = 77/162) and Latinas (26/71). Genotypic distributions for all groups were consistent with Hardy-Weinburg population laws (all p-values fell within the range 0.16-0.91). Interestingly, *HER2*^{V655} allelic frequencies were found to vary with race (African-Americans 0.05, Caucasians 0.16, Latinas 0.15), differences between African-Americans and Caucasians and African-Americans and Latinas were significant (p-values = 0.002 and 0.001, respectively). No association was found between inheritance of *HER2*^{V655} and breast cancer, either in the group as a whole (carriers OR = 1.2, 0.8-1.9 or homozygotes OR = 1.2, 0.4-4.2) or when considered by racial groups or when limited to younger women (<54 years:carriers OR = 1.4, 0.7-2.6 or homozygotes OR = 3.1, 0.7-14.3).

A polymorphism in the human MSH2 gene associated with increased susceptibility to lymphomas. *C. Paz-y-Mino^{1, 2}, J.C. Pérez¹, P.E. Leone^{1, 2}*. 1) Hum Mol Genet & Cytogen Lab., Department of Biological Sciences; 2) Medicine Faculty, Catholic University of Ecuador, Quito-Ecuador.

Infrequent genetic variants include the primary causes of rare genetic diseases, nevertheless, some authors have suggested that the common variants may contribute significantly to genetic risk for common diseases. The central aim of all genetics, therefore, is to correlate specific molecular variation with phenotypic changes. Because of its biological function in mismatch repair, functionally significant hMSH2 gene polymorphisms are candidates for influencing cancer susceptibility and overall genetic stability. We screened for polymorphic variants in the exon 13 of the hMSH2 mismatch repair gene in 72 individuals (50 normal individuals and 22 non-Hodgkins lymphomas) to determine their prevalence in the Ecuadorian population and their probable association with development of lymphomas. The polymorphism screening was realized by Single Strand Conformation Polymorphism (SSCP) analysis. Optimal conditions for SSCP were established and nine samples displaying mobility shifts were identified. These samples were subjected to DNA sequencing. We found a single nucleotide polymorphism: a T to C substitution at the 6 intronic splice acceptor site of exon 13. In the normal individuals group (they have neither disorders nor cancer) the polymorphism frequency was 0.05 (4 individuals, 3 heterozygotes and 1 homozygote), and in the group of lymphomas was 0.11 ($p < 0.01$) (5 heterozygotes). These results suggest that probably the polymorphism is associated with an increased risk to develop non-Hodgkins lymphomas. Because of a former study reported that this allele could also predispose to colon cancer, probably it is associated with susceptibility to different cancers.

Identification and Characterization of Novel Polymorphisms/Mutations in the Glycine N-methyltransferase Gene in Liver Cancer. *T.-L. Tseng¹, Y.-M.A. Chen², J.P. Struewing¹, K. Buetow¹*. 1) Laboratory of Population Genetics, Division of Cancer Epidemiology and Genetic, NCI, Bethesda, MD.20892, USA; 2) Division of Preventive Medicine, Institute of Public Health, School of Medicine, National Yang-Ming University, Taipei, Taiwan 112, R.O.C.

Glycine N-methyltransferase (GNMT) is an enzyme participating in the detoxification pathway, and has been shown to down-regulate cytochrome p4501A1 activity and decrease the DNA adducts formation. Therefore, the expression of GNMT has a protective effect against the exposure of carcinogens. Moreover, GNMT was shown to be diminished in human hepatocellular carcinoma (HCC). We are investigating the GNMT gene for liver cancer predisposition by genotypic and phenotypic characterization of the gene in Taiwanese population and liver cancer cell lines. So far, we have identified several novel mutations and at least five new polymorphisms, including two short tandem repeat polymorphisms and three single nucleotide polymorphisms (SNPs) around flanking region of exon 1. Interestingly, we have identified a novel alternative splice product, skipping exon 1 and its 5'end boundary containing SNPs. Although the allele frequencies are similar in normal individuals and hepatoma patients, the observed frequencies of heterozygosity are significantly different from the expected. We also characterized the genotypes of the GNMT gene in 60 pairs of HCCs. The genotypic distribution between DNA from normal and tumorous liver tissues are the same, however, it is very different in blood and liver DNA. Furthermore, the observed heterozygosity of 2 of the SNPs is dramatically decreased in liver DNA compared with the observed in blood DNA, from 0.31 to 0.09 and 0.5 to 0.15, respectively (p value < 0.005). This may result from the high loss of heterozygosity (LOH) rate within the GNMT gene in liver. Such alterations within the GNMT gene in the liver tissues imply its role in liver cancer development, and the same genotypic alterations in normal and tumorous tissues may suggest evidence of a field effect associated with this locus.

Identification of Lung Cancer Susceptibility Genes After Adjusting for Population Stratification. *Y.-Y. Tsai¹, K.A. McGlynn¹, A.B. Cassidy², Y. Hu¹, J. Arnold², P.F. Engstrom², K.H. Buetow¹.* 1) DCEG/NCI, Bethesda, MD; 2) Fox Chase Cancer Center, Philadelphia, PA.

Population stratification or recent admixture of populations in case-control studies can lead to spurious associations between a phenotype and unlinked candidate loci. We propose an alternative method to account for population stratification by applying nine unlinked, high-heterozygosity STRP markers to assess background relationship among study subjects, and build up logistic regression models to address the effects of candidate cancer susceptibility genes after adjusting for population stratification. A total of 756 smoking individuals, including 462 lung cancer cases and 294 controls, were recruited. Nine microsatellite markers, frequently used in genetic profiling (PE-Biosystems AmpFISTR Profiler Plus), were used as the background information of each subjects. Fifteen genes involved in Phase II metabolism were evaluated. These genes can be categorized into: 1)Epoxide hydrolases; 2)Glutathione S-transferases (GST); 3) N-acetyl transferases; 4)NAD(P)H:quinone oxidoreductase gene. Variants used were either previously described in the literature, or obtained through data mining publicly available sequence data utilizing the SNPpipeline of the NCI's CGAP Genetic Annotation Initiative. To address possibility of population stratification, logistic regression models were performed to evaluate the main effect of 9 markers of DNA profiles between cases and controls, and to examine the independent effect of polymorphisms on cancer susceptibility genes after adjusting for the background. None of the 9 markers of DNA profiles has shown significant differences between cases and controls. After adding susceptibility genes into the model, GST Theta and GSTA4 were significantly associated with lung cancer, with p values of 0.002 and 0.022, respectively. Using logistic regression, we have demonstrated that population stratification was not a serious issue in our data. After adjusting for background markers, we have found that patients who carried both alleles 2 of GSTA4 and those who did not carry any of allele 2 of GST Theta had a significantly increased risk of developing lung cancer.

An association between genetic polymorphisms in the ileal sodium-dependent bile acid transporter gene and the risk of colorectal adenomas^{1s}. *W. Wang¹, S. Xue¹, Q. Chen¹, A.T. Diep¹, A. Stolz², H. Frankl³, R.W. Haile¹*. 1)

Department of Preventive Medicine, Univ Southern California, Los Angeles, CA; 2) Department of Medicine, Univ Southern California, Los Angeles, CA; 3) Kaiser Permanente Sunset, Los Angeles, CA.

Epidemiological and experimental studies have implicated bile acids (particularly, secondary bile acids) as important factors in the development of colorectal cancer. The ileal sodium- dependent bile acid transporter (ISBT) is a crucial player in the enterohepatic circulation of bile acids. Genetic defects in ISBT may result in malabsorption of bile acids and a loss of bile acids into the large intestine, with a resultant increase in the cytotoxic secondary bile acids in the colon produced by bacterial biotransformation. In a case-control study, we investigated the association between two sequence variations in SLC10A2, the gene encoding ISBT, and colorectal adenomas, a precursor lesion of colorectal cancer. The frequency of the missense mutation in codon 171 of exon 3 (a nucleotide transversion from G to T, resulting in an alanine to serine substitution) was not significantly different between cases and controls. However, we found an 85% increased risk of colorectal adenomas associated with a C-> T nucleotide transition in codon 169 of exon 3 (OR=1.85, 95% CI: 1.01-3.37). Logistic regression analysis using A171S/C169T haplotypes as the allelic markers showed that only the haplotype with a T allele at the amino acid position 169 was associated with the increased risk (OR=2.45, 95% CI: 1.28-4.70). The finding of an association between a polymorphism in the SLC10A2 gene and the risk of colorectal adenomatous polyps supports the role of bile acids in the carcinogenesis of colorectal cancer.

BRCA1/2 Testing - Psychological Implications of Informative Vs. Non-Informative Test Results. *S. Eisenberg¹, P. Mor¹, J.S.B. Guedalia², P. Renbaum¹, L. Peled¹, A. Lahad³, E. Levy-Lahad¹.* 1) Medical Genetics, Shaare Zedek Medical Center, Jerusalem, ISRAEL; 2) Neuropsychology Unit, Shaare Zedek Medical Center, Jerusalem, ISRAEL; 3) Department of Family Medicine, Hebrew University, Jerusalem, ISRAEL.

Genetic testing for BRCA1/2 mutations in healthy subjects, has various psychological implications for carriers and non-carriers. For non-carriers, it is important to distinguish between individuals whose familial mutation is known, and those in which the genetic test was non-informative (high risk families where a mutation cannot be identified). For the latter group there is little information on the psychological effects. However, there is a concern that these women may be falsely reassured by negative test results. We examined the anxiety levels and medical behavior (surveillance/preventive actions), in 58 healthy women from high risk families, who received genetic counseling and BRCA1/2 testing, 6 months or more after receiving test results. Women were tested for the three common mutations in Ashkenazi Jews. They were classified into three groups: 1. Carriers. 2. Non-carriers from families with a known mutation. 3. Non-carriers with non-informative test result. We assessed anxiety levels with the Spielbergers State Trait Anxiety Inventory questionnaire, and medical surveillance behavior by a 35-item questionnaire. Multiple regression analysis was used to compare the levels of anxiety between the groups, with group 1 as the reference. Rates of high anxiety were similarly high in groups 1 and 3 (61%), significantly higher than in group 2 (30%, $P=.007$; $OR=.0877$). Interestingly, despite lower anxiety levels, many group 2 subjects continued to follow excessive surveillance routines. Our results show that uninformative negative test results are an independent predictor of anxiety. Levels of anxiety in subjects with non-informative results are as high as those of carriers, and significantly higher than in definite non-carriers. This indicates that these women perceive their risk correctly. In addition, family history influenced medical behavior more than genetic status in many definite non-carriers.

Does genetic counseling for breast cancer predisposition increase knowledge? *K. Brown¹, H. Valdimarsdottir², J. Erlich², D. Amareld³, L. Scheuer³, J. Hull³, D. McDermott³, D. Bovbjerg², K. Hurley², K. Offit³.* 1) Dept Human Genetics, Mount Sinai School of Medicine, New York, NY; 2) Cancer Prevention and Control, Mount Sinai School of Medicine, New York, NY; 3) Dept Human Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY.

An important goal of genetic counseling for cancer predisposition is to improve knowledge about a range of topics, including principles of genetics and oncology, risks for cancer, and options for screening and primary prevention. However, there are little published data on knowledge and comprehension following genetic counseling for breast cancer. Therefore, the major aims of the present study were: 1) to examine the effectiveness of genetic counseling in improving general knowledge about breast cancer/genetics; and 2) to determine if the effectiveness of counseling is related to demographic and psychosocial factors. Participants were 107 women attending individual genetic counseling sessions for breast cancer susceptibility at Memorial Sloan-Kettering Cancer Center in New York. Approximately one week prior to their counseling session, the women completed measures of: 1) breast cancer knowledge (a 27-item questionnaire); 2) cancer specific distress (Impact of Events Scale); and 3) general distress (Profile of Mood States). Approximately one week following their counseling session, the women again completed the knowledge questionnaire. There was a significant increase in knowledge from before to after the genetic counseling session ($p=.0001$). However, there was a wide variability among the women, with no improvement in knowledge among some women. The counseling was less effective for minority women ($p=.007$), less educated women ($p=.05$), and women with high levels of general distress ($p=.003$). When all of these variables were entered together into the equation, ethnicity and general distress remained significant while education was no longer significant. These findings suggest that some women may require different counseling protocols if genetic counseling is to be effective in educating them about their risks and options.

Detection and comparison of telomerase activity in oral squamous cell carcinoma, normal and PHA induced lymphocytes. A.A DELDAR¹, F. MAJIDFAR¹, B. ESLAMI³, H. NAJMABADI², N. MAGHSOODI¹. 1) GENETIC ENGINEERING, RES. CENT. OF SCI. AND BIOTECH, TEHRAN, TEHRAN, IRAN; 2) GENETIC RESEARCH CENTRE, WELFARE AND REHABILITATION UNIV. TEHRAN, IRAN,; 3) DEPARTMENT OF PATHOLOGY, DENTISTRY FACULTY, SHAHID BEHESHTI MEDICAL UNIV., TEHRAN, IRAN,.

Telomerase is an enzyme that maintains telomeres at the end of eukaryotic chromosomes. Activation of this enzyme causes repetition of TTAGGG hexamer which is associated with cell immortalization. Recently the role of telomerase has been considered in human cancers. In this study, activity of telomerase in oral S.C.C. was evaluated and would be followed by detection of telomerase activity in normal and PHA induced lymphocytes. Our research performed on 10 oral S.C.C. paraffin embedded samples out of 200 individuals that refer to our pathology department. We estimate telomeric ends length by PCR that relatively indicate the rate of telomerase activity. In initial report results shown existence of telomeric tails in these abnormal cells. In the malignant cells DNA breaks is observed and seems cells with these changes tend to malignancy. On the other hand telomerase can elongate single strand oligonucleotides whose termini are not complementary to the RNA template sequence 5'-CAACCCCAA-3'. By these data we suggest that presence of variant length of telomeric repeats in cell and PCR products may predict malignancy of these cells.

Simultaneous identification of sequence changes in the p53 tumor suppressor gene and HPV typing using multiplex base excision sequence scanning (M-BESS). *F.W. Hyde, J.T. Schanke.* Epicentre Technologies, Madison, WI.

Recent studies in assessing risk for development of cervical cancer have focused on a mutation in the p53 tumor suppressor gene and its role in the pathogenesis of human papillomavirus (HPV) type 16. Women who have this mutation and are infected with HPV type 16 have been reported to have a higher risk of cervical cancer than those who do not. This finding is the subject of controversy as the results of other studies do not support this conclusion. We examined this correlation with Multiplex Base Excision Sequence Scanning (M-BESS), which allows simultaneous sequence analysis of at least two DNA fragments amplified by PCR. In this method, two DNA fragments are amplified using two different primer pairs labeled with distinguishable fluorescent dyes. The differentially-labeled PCR products are treated with BESS-T and BESS-G Base Reader reagent systems and analyzed using an automated DNA sequencer. We demonstrate the utility of this application in determining the correlation between human papillomavirus (HPV) type and the existence of a specific mutation in codon 72 of the p53 tumor suppressor gene in a single reaction. We demonstrate that M-BESS can simultaneously detect the mutation in codon 72 and type the strain of HPV present.

Completed BRCA1/BRCA2 Mutation Analysis Reveals a Low Rate of Germline Mutation in At-risk African-American Families. *L. Gayol*¹, *H. Basterrechea*¹, *T. Scholl*², *E. Perera*¹, *S. Smith*¹, *J.F. Arena*¹, *L. Baumbach*¹. 1) Univ Miami Med Sch, Miami, FL; 2) Myriad Genetics, Salt Lake City, UT.

The incidence of *BRCA1* germ-line mutations in at-risk individuals is controversial. In Caucasians, the detection of *BRCA1* mutations varies from 5-40%. Even more controversial is the incidence of *BRCA1* mutations and genetic variants in at-risk African-Americans (AA), which has been reported as ranging from very low to incidences equaling those in Caucasians. We report our results of completed *BRCA1* and *BRCA2* analyses in 20 AA families at-risk for breast/ovarian cancer. Families were ascertained based on a history of breast cancer or breast/ovarian cancer and subdivided into three categories: high-risk (HR; three affected 1st degree relatives; 10 families), moderate-risk (MR; two affected 1st degree relatives, 7 families) and undetermined risk (UR; single affected with medical information being updated, 3 families). *BRCA1* and *BRCA2* germ-line alterations were detected first using a series of exon-specific PCR primers for SSCP analysis, followed by DNA sequencing of SSCP variants. A limited number of *BRCA1* polymorphic intronic variants detected as a result of these studies were also analyzed for their effect on *BRCA1* mRNA splicing via an assay developed by Myriad Genetics. In this cohort, only one protein-truncating mutation was detected in either *BRCA1* or *BRCA2* (1/20; 5%). However, splice mutations, missense mutations, and a number of polymorphic variants were detected in both *BRCA1* and *BRCA2*, with a higher frequency occurring in *BRCA2*. Many of these variants were both novel, and specific to AA patients, as well as present in the absence of another disease-causing mutation. Moreover, a novel *BRCA1* missense mutation [exon 19(W1718C)] in one of the HR families appears to co-segregate with breast cancer. The relative frequencies of these *BRCA1* and *BRCA2* variants in patient and control populations will be reported. In summary, these results agree with previous observations that deleterious mutations in *BRCA1* or *BRCA2* are uncommon in at-risk AA patients, and suggest that more benign variants should be further evaluated for their potential role in the disease process in AA patients.

Germline *BRCA2* mutations were detected in individuals from high risk breast cancer kindreds using a novel enzyme mutation detection method. *B.A. Bove, J. Schick, A.T. Yeung, J. Costalas, M.B. Daly, A.K. Godwin.* Fox Chase Cancer Ctr, Philadelphia, PA.

Mutations in the *BRCA2* gene are associated with the development of breast cancer and to a lesser extent ovarian cancer. We have previously reported a highly sensitive enzymatic mutation detection (EMD) method, which relies on a novel plant endonuclease, referred to as CEL I (Oleykowski et al., 1998). CEL I recognizes DNA distortions and cleaves mismatches on the 3-side of the mismatch site in one of the two DNA strands in a heteroduplex. The fluorescent-labeled fragments are resolved by gel electrophoresis and any EMD variants are verified by direct sequencing. We selected 37 females from breast and/or breast/ovarian cancer families attending our Family Risk Assessment Program. Thirty of the individuals selected for screening were diagnosed with breast cancer (29 to 72 yr.), one with ovarian cancer (70 yr.), one with bladder cancer (63 yr.), and five were unaffected. All had previously tested negative for a mutation in *BRCA1* and each reported a family history of at least one first- and/or second-degree relative diagnosed with cancers other than breast and/or ovarian at the following sites: colon (18), stomach (14), lung (13), skin (12), prostate (11), hematopoietic (10), bladder (9), pancreas (7), throat (5), esophagus (5), abdomen (4), bone (4), brain (4), liver (4), kidney (3), rectum (2), gall bladder (2), cervix (1), and oral cavity (1). All 27 exons and intron/exon boundaries of *BRCA2* were evaluated by EMD and mutations were found in 10 of the 37 patients (27%), including six previously unreported mutations. Eight of these mutations were deletions or insertions, one was a splicing variant, and one was a novel missense mutation. Interestingly, all ten *BRCA2* mutation carriers had extensive family histories of cancers in addition to breast and/or ovary. Our results indicate that EMD is a highly sensitive and cost effective method for detecting missense and frameshift mutations in expansive genes such as *BRCA2* and that the incidence of other cancers appears to be elevated in many *BRCA2* mutant kindreds.

Analysis of gene expression patterns in breast cancer by microarray technology. *N. Gokgoz¹, J. Woodget², X. Sun², S. Bull¹, I.L. Andrulis¹.* 1) Samuel Lunenfeld Research Inst, Mount Sinai Hospital, Toronto, Ontario, Canada; 2) Ontario Cancer Institute, Princess Margaret Hospital, Toronto, Ontario, Canada.

Although alterations in several specific genes have been implicated in breast cancer progression, greater understanding of the molecular basis of the disease may benefit from the evaluation of global gene expression patterns using microarray technology. To identify patterns of gene expression that are of prognostic importance in axillary node-negative (ANN) breast cancer, we are taking advantage of a large cohort of ANN patients. Before using the limited RNA from these specimens, we performed pilot studies designed to evaluate the feasibility of using the technology on a larger scale. For this purpose, we studied gene expression in 4 different breast cancer cell-lines (T47D, MDA231, SKBR3 and BT474) using cDNA microarrays containing 1700 and 19000 sequence-verified human cDNAs produced by the microarray facility at the Ontario Cancer Institute, Toronto (<http://www.oci.utoronto.ca/services/microarray>). Each hybridization compared Cy5 labeled cDNA from one of the cell lines with Cy3 labeled cDNA from a reference sample (MCF12A: a normal breast cell line). We also performed reciprocal labeling with subsequent hybridization to demonstrate the consistency and the reproducibility of the technology. In addition, different amounts of RNA (50ug, 25ug and 10ug) from the same cell lines were labeled to determine the sensitivity of the system. We have been able to devise a system that can now be applied to primary breast tumors. After expression analysis, biostatistical modelling will be used to detect clusters of genes that are coordinately expressed and/or repressed. These clusters are likely to represent common pathways of genes involved in breast carcinogenesis.

Absence of MLH3 coding sequence germline mutations in HNPCC families. *S.M. Lipkin¹, V. Wang¹, I. Kirsch², D. Hadley¹, H. Lynch³, F. Collins¹.* 1) GMBB, MSC 4442, NIH/NHGRI, Bethesda, MD; 2) National Cancer Institute, Bethesda, MD; 3) Creighton University, Omaha NE.

DNA mismatch repair is of considerable scientific and medical importance because of its essential role in maintaining genomic integrity, and its association with Hereditary Non-Polyposis Colon Cancer (HNPCC). Linkage analyses originally led to the identification of MLH1 and MSH2 mutations in highly penetrant HNPCC pedigrees. 60-70% of HNPCC families HAVE identifiable mutations in these genes. Subsequently, <1% of HNPCC pedigrees have been found to have PMS2 mutations, and to date only a single PMS1 mutation has been described in one individual from a pedigree with familial cancer clustering. We have recently cloned MLH3, a novel mammalian homologue of yeast DNA mismatch repair gene MLH3p, that appears to be highly expressed in bowel epithelium. In order to evaluate whether MLH3 mutations might underlie unexplained HNPCC, we screened affected probands from more than 40 HNPCC families without identifiable germline mutations in the known candidate genes for MLH3 coding sequence mutations using denaturing HPLC. In no case were we able to identify coding sequence mutations in MLH3 in these probands. Ongoing studies are examining MSI+ colorectal tumors for somatic MLH3 mutations. Although we cannot exclude the existence of rare HNPCC families with underlying mutations, our results suggest MLH3 germline mutations are not frequently associated with HNPCC susceptibility.

Comparison of fluorescence in situ hybridization (FISH) with immunohistochemistry (IHC) for detection of HER2/neu amplification/overexpression in breast cancer. *G. Sun, J.S. Morales, S. Wilz, C. McKee, J. Amberson, S. Gersen.* DIANON Systems, Inc., Stratford, CT.

Adding to its prognostic/predictive value, HER-2/neu is also an indicator for specific therapeutic regimens. However, whether to use immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) or both to assess HER-2/neu status is still a debatable issue. To determine the correlation of IHC and FISH results and thus potentially determine a preferable testing method, 110 consecutive formalin-fixed paraffin-embedded tissue sections of ductal breast carcinoma were studied using the PathVysion FISH assay (Vysis) and the HercepTest IHC assay (DAKO). With the FISH assay, a HER-2/neu to chromosome 17 copy number ratio of 2 or greater was considered positive for gene amplification. With IHC, results of 0 and 1+ did not represent overexpression of HER-2/neu, while 2+ and 3+ were considered positive. Using the FISH assay, HER-2/neu was amplified in 17.3% of samples, while 46.4% demonstrated overexpression with IHC. Seventeen FISH-positive cases had an IHC result of 3+, but three other 3+ cases were negative with FISH. Of 59 IHC-negative cases, only one was positive with FISH. The greatest discordance was encountered in the IHC 2+ patients, where only one of 31 IHC-positive cases was positive with FISH. Conclusions and discussion: 1) Strong (3+) HER-2/neu immunostaining correlates well with gene amplification. 2) FISH is a highly specific and reproducible test for gene amplification. 3) IHC is more cost-effective, and may detect a subset of positive cases that are negative with FISH. 4) Using a mean copy number of HER-2/neu rather than a HER-2/neu to chromosome 17 ratio may reduce the possibility of under-representation of detectable amplified cases. 5) Additional studies are needed for possible improvement of methodology and clinical correlation in IHC 2+ cases.

Extension of the Two-hit Hypothesis in Neurofibromatosis 2 (NF2): Effects of the Mutant Allele and Prediction of the Age of Onset for Both Vestibular Schwannomas. *R.R. Woods^{1,2}, H. Joe^{1,2}, D.G.R. Evans⁴, M.E. Baser⁵, J.M. Friedman^{1,3}*. 1) University of British Columbia, Vancouver, BC, Canada; 2) Department of Statistics; 3) Department of Medical Genetics; 4) St. Mary's Hospital, Manchester, U.K; 5) Los Angeles, U.S.A.

Molecular and epidemiological data are consistent with the application of Knudson's two-hit model for development of vestibular schwannomas (VS) in patients with NF2, but this model does not predict the occurrence of genotype-phenotype correlations or the age at onset of the second tumour in an affected individual. Here we propose some simple extensions to the two-hit models of Moolgavkar and Venzon (Math Biosci 1979; 47; 55-77) and provide an application of these to incidence data for VS in NF2 patients. Our first model, which incorporates an effect of the mutant NF2 allele, was fit to data on 68 NF2 probands with bilateral VS and identified mutation type. Patients were stratified into those with protein truncating mutations (40 patients) and those with other identified mutation types (28 patients). Model-predicted incidence curves for age at onset of hearing loss differed significantly between strata and fit the empirical incidence curves very well for both strata. Our second model combines the techniques used for classical two-hit models with methodology for paired survival data in an effort to predict incidence curves for the ages at onset of both the first and second VS in NF2 patients. Data on the ages at onset for left and right VS for 144 NF2 probands were used to fit the model. Predicted incidence curves from this model fit the empirical incidence curves for the ages of onset for both the first and second VS (See <http://mendel.medgen.ubc.ca/friedmanlab/2hit.html> for plots). A parameter representing the relationship between the ages at onset of the two VS in each patient is estimated in this model and shows a very strong association (Kendall's Tau ~ 0.90).

Audiological phenotype of patients with mitochondrial DNA mutation 3243A>G. *K. Majamaa^{1,3}, S. Uimonen², J.S. Moilanen¹, M. Sorri², I.E. Hassinen³*. 1) Department of Neurology, University of Oulu, Oulu, Finland; 2) Department of Otorhinolaryngology, University of Oulu, Oulu, Finland; 3) Department of Medical Biochemistry, University of Oulu, Oulu, Finland.

The relationship between the phenotype and the genotype is complex in diseases caused by mutations in mitochondrial DNA (mtDNA). The 3243A>G mutation in mtDNA leads frequently to sensorineural hearing impairment (HI), a phenotype that can be measured in severity by audiometry, and consecutive audiograms can give an estimate on the rate of HI progression. We examined the audiological phenotype of 38 patients (14 men, 24 women) with 3243A>G that belonged to the population-based cohort ascertained in the province of Northern Ostrobothnia, Finland. The subjects went through an extensive otorhinolaryngologic examination, including audiometry. Factors modulating the severity of HI were analyzed, and the rate of HI progression was calculated. Better ear hearing level at frequencies 0.5, 1, 2 and 4 kHz (BEHL/0.5-4kHz) was > 20 dB in 28 patients (74 %). A good correlation ($r = 0.550$, $p = 0.001$) was found between BEHL/0.5-4kHz and the degree of the mutant heteroplasmy. BEHL/0.5-4kHz was higher in men than in women, and women outnumbered men among patients with normal hearing or mild HI. In addition, 181 consecutive audiograms were reviewed from 24 patients with HI. The rate of HI progression was calculated to be 2.9 dB/year in men and 1.5 dB/year in women being 5-6 -fold faster compared to the rates that have been observed in the corresponding age group in the general population. High degree of mutant heteroplasmy and male gender were found to increase the severity of HI and its rate of progression. Phenotypic difference by gender may thus be a more universal phenomenon in mitochondrial diseases and not only associated with Leber hereditary optic neuropathy. Furthermore, this study provides the first estimate on the rate of disease progression among patients with the 3243A>G mutation.

RESULTS OF A SCREENING PROGRAM FOR WAARDENBURG SYNDROME IN COLOMBIA. M.L.

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Waardenburg syndrome (WS) is an autosomal dominant disorder characterized by sensorineural hearing loss and pigment abnormalities (white forelock, premature graying, heterochromia irides and skin hypopigmentation). The syndrome is clinically and genetically heterogeneous, and has been divided into four principal types. We search for individuals with Waardenburg Syndrome in the institutions for the deaf in the main cities of our country. Genetic, otological and ophthalmological studies were performed in 1542 individuals with hearing loss, finding a clinical diagnosis of WS in 80 of them (5,2%). A complete medical history and physical examination was performed in all of them and were tested for WI (W Index), cytogenetic examination and molecular screening for the most common mutations. In accordance with our clinical classification we found 45% WS1 and 55% WS2. No WS3 and WS4 were observed. We analyzed 32 WS individuals with high resolution cariotype, showing abnormalities in 4 of them (12.5%). We found: t(13,21)(13pter,13q21::21q22.1-21qter;21pter-21q22.1::1+H25 in one girl, and two different mosaicisms: Normal(60%);t(15q,18ter)(40%) and Normal(96%);t(10,19);10q22.1;19Pter(4%). We also identified a centromeric duplication of chromosome 16 in another girl. As regard of our screening for mutations, we have identified for PAX3 gene 5 mutations: 3 on exon 2, 1 on exon 5 and 1 on exon 6; for MITF gene 3 different mutations on exon 5. Our goal is complete the screening for mutations causing WS, and establish genotype/phenotype correlation in our deaf Colombian population.

Neurological presentation of a congenital disorder of glycosylation CDG Ia : implications for diagnosis and genetic counseling. *V. Drouin-Garraud¹, M. Belgrand¹, S. Grünewald², N. Seta³, J.N. Dacher⁴, A. Hénocq¹, G. Matthijs², V. Cormier-Daire⁵, T. Frébourg¹, P. Saugier-Verber¹.* 1) Service de Génétique, CHU de Rouen, 76031 Rouen, France; 2) Center for Human Genetics, University of Leuven, B 3000 Belgium; 3) Laboratoire de Biochimie, CHU Bichat-Claude Bernard, 75877 Paris; 4) Service de Radiologie, CHU de Rouen, 76031 Rouen; 5) Département de Génétique, Hôpital Necker Enfants-Malades, 75743 Paris, France.

The congenital disorders of glycosylation type I (CDG I) are rare autosomal recessive disorders characterised by a defective glycosylation of complex glycoconjugates. CDG Ia, the most frequent type, is a multisystemic disorder affecting the nervous system and numerous organs including liver, kidney, heart, adipose tissue, bone and genitalia. A phosphomannomutase (PMM) deficiency has been identified in CDG Ia patients and numerous mutations within the *PMM2* gene, located on chromosome 16p13, have been recently identified in patients with PMM deficiency. We report here a French family including 3 affected sibs, with an unusual presentation of CDG Ia remarkable by (i) the neurological presentation of the disease and, (ii) the dissociation between a subnormal PMM activity in fibroblasts and a decreased PMM activity in leukocytes. This case report shows that the diagnosis of CDG Ia must be considered in patients with non-regressive early-onset encephalopathy with cerebellar atrophy and that intermediate values of PMM activity in fibroblasts do not exclude the diagnosis of CDG Ia.

A Korean patient with Fanconi-Bickel syndrome(FBS) presenting with transient neonatal diabetes mellitus and galactosemia. *J.W. Cheong¹, G.H. Kim², E.J. Seo², H.W. Yoo².* 1) Asan Institute for Life Sciences, Asan Medical Center, Seoul, Korea; 2) Medical Genetics Clinic & Laboratory, Asan Medical Center, Ulsan University College of Medicine, Seoul, Korea.

Fanconi-Bickel Syndrome(FBS) is a rare autosomal recessive disorder of carbohydrate metabolism recently demonstrated to be caused by mutations in GLUT2, the gene for the glucose transporter protein 2 expressed in liver, pancreas, intestine and kidney. This disease is characterized by hepatorenal glycogen accumulation, both fasting hypoglycemia as well as postprandial hyperglycemia and hypergalactosemia, and generalized proximal renal tubular dysfunctions. We report the first Korean patient with FBS diagnosed based on clinical manifestations and identification of novel mutation in the GLUT2 gene. She was the first full term product born to normal healthy parents with non-consanguineous marriage. She was initially diagnosed as having a neonatal diabetes mellitus due to hyperglycemia and glycosuria at 3 days after birth. Newborn screening for galactosemia also revealed hypergalactosemia. Galactose-1-phosphate uridylyltransferase activity was within normal range. Thereafter, she has been managed with lactose free milk, insulin therapy. However, she failed to grow and her liver has been progressively enlarged. Her cheeks were chubby, and her abdomen was protruded. Laboratory tests indicated she had generalized proximal renal tubular dysfunction; renal tubular acidosis, hypophosphatemic rickets, generalized aminoaciduria. Given aforementioned findings, the diagnosis of FBS was entertained at age of 2 months. Her genomic DNA was isolated from peripheral leukocytes. The nucleotide sequencing of the GLUT2 gene from the patient revealed a novel mutation; K5X/K5X.

Evidence for a novel X-linked adrenal insufficiency with salt wasting. *E.Robert Wassman¹, M. Moline^{1,2}, S. Clark², M. Ibrahim³, J. Siegel-Bartelt,¹*. 1) PAMG, Pasadena, CA; 2) Pomona Valley Hospital; 3) San Antonio Community Hospital.

Genetic heterogeneity is reported for many conditions. We report novel X-linked inheritance of congenital adrenal insufficiency with salt wasting in a family with 3 affected males originally thought to have 21 hydroxylase deficiency. Three obligate carrier females are two maternal half sisters and their mother. The family resides in a large metropolitan area, and there is no consanguinity. The oldest affected child is a now 16 year old male who was found to have dehydration, hyperpigmentation, and salt wasting at 2 weeks of age. He carries a diagnosis of 21 hydroxylase deficiency, and is maintained on hydrocortisone and fludrocortisone. Commercial molecular analysis of the steroid 21-hydroxylase gene (HACHapman Institute) revealed no G mutation, nor 8bp mutation characteristic of a gene deletion in the CAH gene or pseudogene conversion, nor point mutations M239K, V281L, Q318X and R356W. Case 2 is a now 4 year boy born to the proband's maternal half sister. He also presented at 12 days with dehydration and salt-wasting, and is being maintained on Cortef and Florinef. His course has been complicated by hypertension, and he has an elevated 11-deoxycortisol. Case 3 is a one year old male with similar presentation born to another maternal half sister to the proband. This pedigree is highly suggestive of previously undescribed X-linked inheritance of congenital adrenal hyperplasia with salt wasting, as the odds of 3 unrelated carriers marrying into the family is low. We speculate that an X-linked factor modifies this steroid synthesis pathway. Genetic counseling in families with males probands should include this possibility of genetic heterogeneity.

Proximal 1q deletion syndrome: A patient with del(1)(q24.2-q25.3). *M. Descartes*¹, *J. Zenger-Hain*², *M. Conklin*³, *A. Carroll*¹. 1) Dept Human Genetics, Univ Alabama, Birmingham, AL; 2) Oakwood Hospital, Dearborn, MI; 3) Pediatric-Orthopedic Children's Hospital, Univ. of AL, Birmingham, AL.

Patients with deletion 1q21-q25 have characteristic features that have allowed the delineation of a syndrome, proximal 1q deletion syndrome.¹Based on 11 reported cases, the features include growth retardation, microbrachycephaly, psychomotor retardation, hypertelorism, abnormal ears, bulbous nose, micrognathia, cleft lip/palate, small hands/feet, 5th finger clinodactyly, dysplastic nails, hernias, genital and cardiac anomalies.¹⁻⁵We report on a 13 year old female patient with a smaller deletion than previously described. Her karyotype was 46,XX,del(1)(q24.2-q25.3)*de novo*. Our patient had many of the previously described features as well as, partial growth hormone deficiency, small optic nerves, and hearing loss. The later features have been described in patients with more distal deletions, i.e. del(1)(q24q32).⁶⁻⁷ Her physical examination revealed microcephaly, short forehead, flared eyebrows, bulbous nose, flared nares and broad columnella, posteriorly rotated ears, full lips and small chin. In addition, bilateral pes cavus, bowing and shortening of the forearms with Madelung's deformity, limited pronation and flexion of wrists and elbows, short fingers and toes, broad thumbs, and 5th finger clinodactyly were noted. The patient attends an educable mentally impaired school program. Radiographic examination revealed: 11 pairs of ribs; relative ulnar shortening and subluxation; shortened/broadened phalanges; 4-5th metacarpals with abnormal shape; fusion of the distal phalanges; short 3-5th metatarsals. She did not have cleft lip/palate, genital or cardiac defects. This case narrows the critical region for the proximal 1q deletion syndrome, and a possible candidate is T-box19 which maps to 1q23q24.⁸ Mutations in other genes in the T-box family have been implicated in multiple malformation syndromes in which skeletal anomalies are the features such as Ulnar-Mammary syndrome (TBX3)⁹ and Holt-Oram syndrome (TBX5).¹⁰.

A Lethal Skeletal Dysplasia - Distinct from Neu-Laxova Syndrome. *A.M. Elliott¹, J. Hoeffel², M. Gonzales³, R.S. Lachman¹.* 1) International Skeletal Dysplasia Registry, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Hôpital Jeanne d'Arc, Toul Cedex, France; 3) Hôpital St-Antoine, Paris, France.

Neu-Laxova Syndrome (NLS) is a severe disorder with intrauterine growth retardation, edema and characteristic facies (including microcephaly with receding forehead, protuberant eyes, a flattened nose, deformed ears, cleft palate and micrognathia). Ichthyosis is often present. Limb anomalies include hypoplastic fingers and syndactyly of fingers and toes. Patients are usually stillborn or die shortly after birth. We report two unrelated patients with clinical findings suggestive of Neu-Laxova syndrome, but with anomalies not usually seen in this disorder. These patients likely represent a distinct lethal skeletal dysplasia. Both patients were stillbirths. Patient 1 was a stillbirth at 24 weeks gestation and is of North African descent. Radiographic evaluation of patient 1 revealed vertebral body deficiency with a short trunk. The clavicles were elongated. There appeared to be 11 pairs of ribs which were proportionately thickened. The skull was large. There was rhizo-, meso- and acromelia. There was hypoplasia of the metacarpals and phalanges. The feet were similarly affected. The ilia were tall and narrow and the sacrosciatic notches were widened. The ischia were vertical. The long bones showed diaphyseal widening which spared the metaphyses and was more pronounced in the lower extremities. Patient 2 showed similar radiographic changes with a large skull, short trunk, tall and narrow ilia and vertical ischia. The hips were dislocated and the acetabular region never formed. The diaphyseal bony overgrowth primarily involved the lower extremities. Clinically, there was macrocephaly, short limbs with hypoplastic digits and generalized edema. Scott et al., (Am J Med Genet 9:165-175, 1981) described 2 patients with Neu-Laxova syndrome with radiographic and clinical findings similar to the 2 patients reported here. Scott's patients were described as having short-limbs, large heads, short necks and hypoplastic/absent digits and generalized edema. These 4 unrelated patients lack the typical findings of NLS, and likely represent a distinct lethal skeletal dysplasia.

Bone mineral metabolism in Satoyoshi syndrome associated with autoimmune thyroiditis. *V. del Castillo¹, N. Altamirano¹, A. Rasmussen², C. Esmer¹, M. Ruiz¹.* 1) Inst Nacional Pediatria, SSA, Mexico, DF; 2) Inst Nacional de Neurologia y Neurocirugia, SSA, Mexico, DF.

Satoyoshi syndrome is a rare disorder characterized by progressive muscle spasms, alopecia, diarrhea, skeletal abnormalities and endocrine disorders. The frequency and severity of spasms slowly increases causing skeletal damage secondary to repeated traumas, manifested as metaphyseal lesions, slipping of epiphyses, cystic lesions, acro-ostelolysis, bone fragmentation, fatigue fractures and early osteoarthritis. Other common features are: amenorrhea, mild microcytic anemia, short stature and impaired glucose tolerance. Although etiology is unknown, an autoimmune mechanism has been proposed. We report on a 14-year-old female that experienced painful muscle spasms in the lower limbs at age 6 years with progressive involvement of the upper limbs, trunk, neck and masticatory muscles, with articular deformity that led to difficult walking. Soon after, she had alopecia involving scalp hair, eyebrows and eyelashes, and intermittent diarrhea. Her radiologic findings are those typically described in the syndrome. Femoral neck and lumbar spine L1-L4 bone density was decreased and biochemical markers of bone turnover (serum Ca, P, Mg, alkaline phosphatase and Ca/Cr ratio) were within normal limits. She was responsive to dantrolene and biphosphonate administration during 20 months in which autoimmune thyroiditis was diagnosed and managed with thyroid hormones. Dantrolene was suspended one year ago. At the present time, antithyroid antibodies are negative, bone density has improved, new hair has grown and the muscle spasms have decreased in frequency and intensity, she has Tanner 3 breast development, but menarche is still absent. To our knowlegde this is the first case who shows abnormal bone density succesfully treated with biphosphonates. Also is the first in which thyroiditis is described, this finding provides support to the autoimmune theory.

Pre-Expression Evaluation of Spondyloepiphyseal Dysplasia Tarda By Sedlin Gene Analysis. *G.S. Gottesman^{1,2}, S. Mumm³, X. Zhang³, W.H. McAlister⁴, M.N. Podgornik¹, M.P. Whyte^{1,3}*. 1) Metabolic Research Unit, Shriners Hosp Children, St Louis, MO; 2) Div Medical Genetics, Saint Louis Univ Sch Medicine, St Louis, MO; 3) Div Bone Mineral Diseases, Wash Univ Sch Med, St Louis, MO; 4) Dept Radiology, St Louis Childrens Hosp, St Louis, MO.

Spondyloepiphyseal dysplasia tarda (SEDТ) is a X-linked recessive disorder that presents during childhood with back pain and short stature in boys. As we have reported (Medicine 78:9-25,1999), heterozygous women can manifest subtle radiographic changes. However, skeletal radiographs of affected (hemizygous) boys are normal during infancy and early childhood. In 1999, 2 bp deletions were identified in a gene designated Sedlin in 3 Australian families with SEDТ (Nat Genet 22:4, 400-404 1999). Although there is no established medical treatment for this condition, pre-expression diagnosis by sedlin gene analysis could be beneficial, allowing appropriate counseling against physical activities injurious to the spine and the joints, especially those of the lower limbs.

After an orthopedist referred a 15-year-old young man (proband) with dysplastic changes of the spine, we established a diagnosis of SEDТ. He was from a large kindred with previously undiagnosed SEDТ involving at least 5 generations. A unique 2 bp deletion in exon 5 was found on molecular analysis by PCR amplification and sequencing of all the coding exons. We then studied a nuclear family from within the kindred (proband's maternal aunt and her 3 sons). The woman was a potential carrier. Subtle shaping abnormalities of her pelvis and knees on skeletal radiographs supported this possibility. Radiographs of the spine and pelvis of the 2 younger boys (ages 2 and 5) showed no changes of SEDТ, but those of the eldest son (age 8) had clear-cut evidence of the disorder. Molecular testing demonstrated the mother and only her eldest son had the same 2 bp deletion in exon 5. Molecular testing for Sedlin gene abnormalities enables pre-symptomatic diagnosis prior to radiographic demonstration of the disorder. Perhaps, cautions early on to minimize physical strain to the spine and joints will ameliorate the eventual manifestations of the disorder.

Ophthalmoplegia, severe scoliosis and brain stem hypoplasia: A recognized autosomal recessive syndrome. *B. Lo¹, S. Blazer², B. Banwell³, N. Quercia¹, A. Teebi¹.* 1) Division of Clinical Genetics, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Division of Diagnostic Imaging, Hospital for Sick Children, Toronto, Ontario, Canada; 3) Division of Neurology, Hospital for Sick Children, Toronto, Ontario, Canada.

Congenital horizontal gaze palsy associated with early onset severe scoliosis is a rare disorder that has been hypothesized to be due to brainstem maldevelopment. In families previously ascertained, however, brain imaging studies have not uncovered any brainstem abnormalities, and intelligence has been reported as normal or borderline. We describe here a consanguineous family in which three of five siblings have congenital horizontal gaze palsy and early onset severe scoliosis. The affected siblings exhibit complete ophthalmoparesis of the medial and lateral recti muscles bilaterally with relatively well preserved superior and downward gaze, and scoliosis of the thoracolumbar spine requiring either surgical repair or bracing. In two of the affected siblings, a brother and sister, there was also significant cognitive delay. Interestingly, MRI scans revealed brainstem hypoplasia in these two individuals with cognitive delay. MRI on the sister, age 12, demonstrated a prominent midline raphe of the medulla and an abnormal flattened configuration of the pons. Visual and auditory evoked potentials, EMG and nerve conduction studies performed on the brother, age 6, were all within normal limits. A muscle biopsy taken from the sister did not provide any evidence for a mitochondrial or structural myopathy and karyotyping of the brother was unremarkable. The siblings did not exhibit any significant dysmorphic features. The MRI results in this family provide support for the theory that the maldevelopment of neurons in the abducens nuclei and caudal longitudinal fascicle is the cause for this rare syndrome of horizontal gaze paralysis and kyphoscoliosis.

A female case of osteopathia striata with cranial sclerosis associated with markedly skewed X-chromosome inactivation: Implication of an X-linked trait. *Y. Nozaki¹, T. Kubota², Y. Fukushima², T. Yamagata¹, M. Mizuguchi¹, M.Y. Momoi¹, G. Nishimura³.* 1) Pediatrics, Jichi Medical School, Minamikawachi, Tochigi, Japan; 2) Department of Hygiene and Medical Genetics, Shinshu University School of Medicine, Nagano, Japan; 3) Department of Radiology, Nasu-Chuo Hospital, Tochigi, Japan.

Osteopathia striata with cranial sclerosis (OS-CS) is a sclerosing bone dysplasia associated with a variety of dysmorphic features and mental retardation. OS-CS has been considered to be dominantly inherited. However, female preponderance and male severity in OS-CS have raised a question as to whether this entity is transmitted as an autosomal dominant or X-linked trait. We report here a female patient with OS-CS with some additional features. The parents of the patient were healthy, and the mother had two miscarriages. The clinical manifestation of the patient consisted of macrocephaly with frontal bossing, hypertelorism, broad nasal bridge, cleft palate, cataract, and severe mental retardation. The radiologic manifestations of the patient, including longitudinal striation of the metaphyses of long bones and sclerosis of cranial bones, warranted a diagnosis of OS-CS. In addition, she had severe conspicuous brachydactyly, scoliosis, features rarely described in OS-CS. To elucidate the pathogenesis of OS-CS in this female patient, we studied the inactivation pattern of X-chromosomes on lymphocytes by methylation-specific PCR. The DNA of the patient and parents was treated with sodium bisulfite that converts all unmethylated, but not methylated, cytosines to uracil. PCR primers were designed to detect active and inactive X chromosomes separately by utilizing repeat length polymorphism of PCR products. The patient had a 213 bp band from the father and a 195 bp band from the mother with as much as 97% activation of the mother-derived X-chromosome. These results suggest that in this patient OS-CS had been inherited in an X-linked fashion and became manifest due to extremely skewed inactivation of X chromosomes.

Neonatal demise in an infant with a molecular diagnosis of osteogenesis imperfecta Type I. *V. Loik Ramey¹, J.G. Habecker-Green¹, G. Markenson², S. Pflueger³, G.M. Cohn¹.* 1) Clinical Genetics, Baystate Medical Center, Springfield, MA; 2) Maternal-Fetal-Medicine, Baystate Medical Center, Springfield, MA; 3) Pathology, Baystate Medical Center, Springfield, MA.

Osteogenesis imperfecta Type I is the mildest form of OI and is not typically associated with neonatal demise. We present a female infant with a molecular diagnosis of OI Type I who had multiple congenital anomalies and expired at 22 hours after birth.

The infant was diagnosed prenatally with an isolated congenital heart defect of undefined type. The pregnancy was significant for poorly controlled diabetes. The parents were maternal first cousins as were their maternal grandparents. The infant, born at 33 weeks gestation, sustained a femur fracture during delivery. Postnatally, she sustained a humeral fracture without trauma. The evaluation of the infant was notable for markedly redundant and hyperextensible skin. Dysmorphic features included: a bulbous nasal tip, retrognathia, anomalous ears, ambiguous genitalia, dorsiflexed left foot with hyperflexion of toes, and clenched fists. A skeletal survey revealed multiple prenatal rib fractures with otherwise unremarkable ribs and long bones. The skull was significant for wormian bones, undermineralization, and calvarian fractures. The infant expired 22 hours after birth secondary to cardiorespiratory failure. Autopsy revealed a hypoplastic transverse aortic arch and main pulmonary artery dilation with elongated tortuous right and left pulmonary arteries. Collagen studies revealed a functional null allele for COL1A1 consistent with a clinical diagnosis of OI Type I. Heterozygosity studies ruled out a microdeletion of chromosome 17q.

There have been no reports in the literature describing a case of OI type I with the severity of clinical symptoms seen in this infant. Whether this case represents a severe form of isolated OI Type I or OI Type I in combination with another disorder remains a question. As the parents were multiply consanguineous, we hypothesize that this infant may have also had another autosomal recessive connective tissue disorder.

A new connective tissue disorder with features overlapping those of Ehlers-Danlos, Marfan and Stickler

syndromes. *H.P. Levy¹, J.J. Johnston¹, J. Davis¹, J. Balog¹, P.S. Rose^{1,2}, A. Schäffer¹, H. Dietz², C.A. Francomano¹.*
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A family with manifestations overlapping Ehlers-Danlos, Marfan and Stickler syndromes originally came to medical attention when a 58 year old mother and 32 year old son both died from spontaneous aortic dissection. The mother's father and two uncles also died from vascular dissections (two cerebral and one abdominal aneurysm). We have examined thirteen living relatives (nine affected). Common features among affected individuals include joint laxity and mild skeletal abnormalities, which are nonspecific for a particular connective tissue disorder. Vascular dilatation and dissection are most consistent with a diagnosis of Marfan syndrome. Findings suggestive of Ehlers-Danlos syndrome include soft, fragile skin and a bleeding diathesis. Many individuals also have characteristics typical of Stickler syndrome, including premature osteoarthritis, bifid uvula (a form fruste of cleft palate), hearing loss, and vitreoretinal degeneration. In addition, there may be progressive microretrognathia, similar to but more severe than that seen in Marfan and Stickler syndromes, and profoundly simple auricular helices. Peripheral blood karyotype and biochemical analysis of types I and III procollagen and collagen from fibroblasts were normal. A full genome scan was performed using a linkage set with 10 centimorgan spacing. LOD scores for markers surrounding COL11A2 exceeded -2 and were negative for markers flanking FBN1, FBN2, COL1A1, COL1A2, COL2A1, COL3A1, COL5A1 and COL5A2. Power was too low to confirm linkage to any particular region, and efforts are underway to include additional family members and analyze additional markers.

In summary, we describe a new autosomal dominant connective tissue disorder with features overlapping those of Ehlers-Danlos, Marfan and Stickler syndromes. No single defined heritable disorder of connective tissue reconciles all of the manifestations, and preliminary data appear to exclude the majority of genes associated with these disorders.

A man who inherited SRY and Leri-Weill dyschondrosteosis from his mother and neurofibromatosis from his father. *F. Wei¹, S. Chheng¹, F.F.B. Elder¹, C.I. Scott Jr.², L. Nicholson², J.L. Ross³, A.R. Zinn³.* 1) McDermott Center for Human Growth and Development, University of Texas Southwestern Medical School, Dallas, TX; 2) Alfred I. duPont Hospital for Children, Wilmington, DE; 3) Department of Pediatrics, Thomas Jefferson University, Philadelphia, PA.

CASE REPORT: We studied a 29 year old man with Leri-Weil dyschondrosteosis (LWD) and neurofibromatosis (NF). He was 146 cm. tall (-4.6 SD) and had Madelung's deformity, mesomelia, café au lait spots and neurofibromata. His father was 162 cm. tall (-2.3 SD) and had NF. His mother was 142 cm. tall (-3.4 SD) and had Madelung's deformity, mesomelia, high arched palate, bicuspid aortic valve, aortic stenosis, and premature ovarian failure at age 40 after two successful pregnancies. A male sibling had NF but not LWD.

RESULTS: Karyotype of the mother for suspicion of Turner syndrome showed mos 45,X[17 cells]/46,X,dic(X;Y)(p22.3;p11.32)[3 cells].ish dic(X;Y)(DXZ1+,DYZ1+). The proband's karyotype was 46,X,dic(X;Y)(p22.3;p11.32). Additional studies showed that the dic(X;Y) chromosome was PABY+, SRY+, DYZ5+, and SHOX⁻. The dic(X;Y) chromosome was also positive for a marker < 300 kb distal to PABX, suggesting that the translocation deleted only pseudoautosomal sequences, including SHOX.

DISCUSSION: Haploinsufficiency of SHOX explained LWD in the proband and his mother. Her female phenotype could be due to 45,X mosaicism, SRY silencing by X-inactivation, or both. X-inactivation studies of the proband and his mother are in progress. The proband was normally virilized; detailed andrologic evaluation is planned. Prophylactic oophorectomy was recommended for the mother because of the risk of gonadoblastoma. This family segregating multiple Mendelian and chromosomal disorders demonstrates remarkable sex chromosome variation compatible with grossly normal male and female sexual differentiation. The case also highlights the importance of a karyotype for differentiating Turner syndrome and LWD, especially in patients with findings that are not explained by SHOX haploinsufficiency, such as premature ovarian failure or aortic abnormalities.

The "Marshall Chronicles:" Splicing mutation of 54-bp exon in the COL11A1 gene not found in four generation family with Marshall syndrome (MS)(MIM 154780). *A.L. Shanske¹, J. Korkko², R. Marion¹.* 1) Ctr Congenital Disorders, Children's Hospital at Montefiore, Albert Einstein College of Medicine, Bronx, NY; 2) Center for Gene Therapy, MCP Hahnemann University, Philadelphia, PA.

In 1958, Marshall described 7 members in 3 generations of a family who were affected with a form of "ectodermal dysplasia" comprising ocular abnormalities, minor facial anomalies and hearing loss. The author emphasized the ectodermal and ocular abnormalities. Since the original description, there have been only 8 additional families including our own (Shanske, 1997). Since then, nosologic relationships among MS, Stickler Syndrome (SS) and a number of related disorders have been debated. The recognition of molecular defects in patients with SS has revealed genetic heterogeneity but has not resolved the molecular basis of MS.

Clinical Reports: The propositus, EM is a 21 year old woman with midfacial hypoplasia, telecanthus, broad flat nasal bridge, sparse scalp hair, lashes and brows and normal sweating. She has myopia, exotropia and embryotoxin. She underwent craniofacial reconstruction consisting of orbital translocation, cranial bone graft, first stage nasal reconstruction and medial and lateral canthopexy. FM is the 18 year old brother of EM with the identical craniofacial and ectodermal findings and has also undergone craniofacial correction of hypertelorism. He has high myopia and bilateral amblyopia. NM is their 14 year old brother who also underwent a craniotomy to repair third degree hypertelorism. He has myopic astigmatism and exotropia. EM's mother, maternal grandfather and 2 of her 3 children have the same phenotype.

PCR amplification of the exons and the exon-intron boundaries of the 54 exons of COL2A1 and the 67 exons of COL11A1 was followed by screening of the PCR products by the technique of confirmation gel electrophoresis. No unusual sequence variations were identified in NM. We conclude that neither a splicing mutation of 54-bp exon in the C-terminal region of COL11A1 nor the several mutations identified in COL2A1 in patients with the MS/SS phenotype are the molecular basis of MS in this family.

Presumed AR ulna/fibula dysplasia with cleft lip: a third reported family. *J. Siegel-Bartelt*^{1,2}, *M. Chung*³, *E.R. Wassman*^{1,2}, *G.R. DeVore*¹. 1) Alfigen, The Genetics Inst, Pasadena, CA; 2) Perinatal Alliance Medical Group; 3) Glendale Adventist Hospital.

We report a sibship with 3 affected children with ulna/fibula dysplasia similar to the families previously reported by Pfeiffer, RA (1988) and Fuhrmann, W (1980.) This supports a rare autosomal recessive etiology for the ulna/fibula developmental field. The limb anomalies in the proband were seen at 26 weeks on ultrasound. The term AGA male had poor respiratory effort, and died at 9 hours. There were short angulated forearms, with ulnar clubbing of the hands. The ulna was markedly hypoplastic, 4 metacarpals were present, and 4 digits. The thumbs were also smaller than usual. The lower leg was slender with a short fibula, 4 metatarsals, and 4 digits with 1,2 syndactyly. The face showed hypertelorism, incomplete left cleft lip, small mouth with trismus, and small lowset ears. The chest was broad, with partial superior duplication of the nipple. The genitalia were hypoplastic. Chest Xray showed an enlarged heart. Chromosome analysis was normal, 46,XY. The family history revealed normal parents (not known to be consanguineous but from adjacent rural villages.) The first affected child was a female who died at 6 days. She had 3 digits on the hands and feet, and a mouth that did not open. The second child in the family is a male with 4 digits on each extremity. He is alive at 6 years with developmental handicap (walked at 3 years.) He had prolonged gastrostomy feeding, and had infant heart surgery. His facies are similar to the proband, with hypertelorism, lowset cupped ears, and mild retrognathia. The ulna/fibula developmental field is most frequently affected in sporadic birth defects, such as Femur-fibula-ulnar syndrome. However, it is also affected in a few autosomal dominant conditions, such as Schinzel ulnar-mammary syndrome due to TBX3 at 12q24, and Weyer acrodistal dysostosis due to EVC gene at 4p16. Kaplan fibulo-ulnar aplasia with renal anomalies is also presumed to be autosomal recessive. This case report supports the concept of a fibulo-ulnar developmental field with heterogeneous etiologies.

Additional Skeletal Features in Pallister-Hall Syndrome. *T. Roscioli¹, D. Kennedy², G.F. Watson³, B. Fonseca⁴, J. Pereira⁵, D. Mowat¹*. 1) Dept Medical Genetics, Sydney Children's Hospital, Sydney, Australia; 2) Central Sydney Clinical Genetics Service; 3) Department of Anatomical Pathology, Royal Prince Alfred Hospital; 4) St. George Hospital Paediatric Unit; 5) Department of Radiology, Sydney Children's Hospital.

Pallister-Hall Syndrome (PHS) is a dominant condition comprising insertional polydactyly, hypothalamic hamartoma and anogenital abnormalities. Mutations of the GLI3 zinc finger protein have been found in PHS, Greigs Cephalopolysyndactyly and Polydactyly type A. We present two isolated cases of PHS; one a fetus and one a neonate. In the former, routine 18 week antenatal ultrasound showed 3-4 finger syndactyly, shortening of both lower limbs with fibular hypoplasia and asymmetrical radio-ulnar shortening. Examination post mid-trimester induction showed a female fetus with bilateral postaxial and insertional polydactyly, left 3-4 syndactyly, a bifid right middle finger and short, bowed forearms. Bilateral foot post-axial polysyndactyly, mesomelic lower limb shortening and an imperforate anus was present. An autopsy showed a rectovaginal fistula, vaginal atresia and a hypothalamic hamartoblastoma. X-rays showed mesomelic changes in the ulna, tibia and fibula with blunted metaphyses and decreased epiphyseal ossification. The second case was diagnosed after birth with bilateral lower limb acromesomelia, bilateral brachydactyly, insertional left third toe polydactyly, variable syndactyly, digital nail hypoplasia, and an anteriorly placed anus with stenosis. Investigations confirmed the presence of a hypothalamic tumour, growth hormone deficiency, bilateral renal dysplasia, a laryngeal cleft, epiglottal hypoplasia and a vascular ring. X-Ray findings included bilateral hip dysplasia, shortened tibias, proximal syndactyly of the left 3-4 metacarpals and insertional polydactyly of the left 2nd and 4th toes. These two cases have classical features of PHS as well as a generalised skeletal dysplasia with acromesomelic limb shortening not highlighted in previous reports. PHS should be included in the differential diagnosis of antenatally ascertained mesomelic limb shortening particularly in the presence of polysyndactyly.

Female-to-male transmission of isolated Periventricular Nodular Heterotopia, cosegregating with the Xq28 region. *M. Gerard-Blanluet¹, K. Machinis², C. Danan¹, J. Patkai¹, A.S. Trentesaux¹, M. Benani¹, M. Sinico¹, F. Encha-Razavi¹, C. Walsh³, P. Brugieres⁴, M. Goossens², J.C. Janaud¹, S. Amselem².* 1) CHIC Creteil, France; 2) Genetique Moleculaire, Hopital Henri Mondor, Creteil, France; 3) Neurology, Harvard Medical School, Boston USA; 4) Neuroradiologie, Hopital Henri Mondor, Creteil, France.

We report the first female-to-male transmission of periventricular nodular heterotopia. Periventricular heterotopia is an OMIM listed genetic disease (OMIM number 300049), described in 1993. The disorder, which is usually transmitted as an X-linked dominant trait, is lethal in males. MRI images can mimic Bourneville syndrome, but clinical features show no mental retardation, no depigmented skin patches, and partial seizures, occurring usually in late infancy. The Filamin-1 gene is located at Xq28, spans 47 exons, and was proved to be responsible for this disorder. Two dizygotic diamniotic twin boys were born prematurely at 26 weeks because of chorioamniotic infection. The mother is 23 and the father is 41 years old. There is no family history of epilepsy, nor abnormal spontaneous abortions. The twin boys were eutrophic, without external malformations. They both have large anterior fontanels. Periventricular wall irregularities were noted in each twin, the first day of life. MRI, done at three months of age for both, revealed multiple periventricular nodular heterotopia. EEGs were normal. Developmental milestones seemed normal. Maternal MRI showed asymptomatic nodular periventricular heterotopia. Cytogenetic analysis, done with a peculiar attention at the Xq28 region, was normal, as metabolic screening. Molecular analysis demonstrated that the twins are dizygotic. Each has inherited a different X chromosome. They share the same Xq28 locus, spanning the Filamin-1 region, because one of the twin had a recombined X chromosome. Mutations in Filamin-1 gene are then actively searched for. As far as we are aware, this observation demonstrates the first female-to-male transmission of periventricular nodular heterotopia, possibly linked to the Filamin-1 gene. A mild Filamin-1 mutation may explain the viability in males.

CUTIS LAXA, CHARACTERISTIC FACIAL DYSMORPHISM, CLEFT PALATE AND MENTAL RETARDATION: A NEW SYNDROME? *D. Genevieve¹, L. Faivre¹, C. Baumann², D. Sanlaville¹, S. Jacquemont¹, C. Bodemer³, S. Lyonnet¹, A. Munnich¹, V. Cormier-Daire¹.* 1) Departement de Genetique, Hopital Necker Enfants malades, Paris, FRANCE; 2) Service de Neonatologie, Hopital Robert Debre, Paris, FRANCE; 3) Service de Dermatologie, Hopital Necker Enfants Malades, Paris, FRANCE.

We report on the association of cutis laxa, facial dysmorphism, cleft palate and moderate mental retardation in 3/7 children born to first cousin healthy parents. Two girls and a boy presented with cutis laxa, increased joint laxity and facial dysmorphism including flat and round face, very short nose, sagging cheeks, blue sclerae, hypertelorism and small chin. Additional features were hygroma in early pregnancy (2/3), hard cleft palate (2/3) and ventricular septal defect (2/3). The boy died at 25 day of life of progressive emphysema and multiple pneumothorax. In the girl, skin abnormalities and developmental delay improved in the course of the disease. Skin histology showed a lack of elastic fibers in 2/3 cases. X-rays, ophthalmologic examinations and blood chromosomes were normal. Autosomal recessive cutis laxa type II could be considered because of the association of cutis laxa, mental retardation and joint laxity but facial dysmorphism is unusual in this syndrome. Emphysema in one patient was also suggestive of autosomal recessive cutis laxa type I, but the other clinical features did not fit with this syndrome. Our patients share clinical features with the spectrum of wrinkly skin syndrome, namely cutis laxa and mental retardation but absence of growth delay and of wrinkled skin over the dorsum of hands and feet of our patients rules out this diagnosis. We propose therefore that this distinctive association represents a novel entity of autosomal recessive inheritance.

Microcephaly with jejunal atresia, a possible new syndrome. *D.N. Abuelo¹, W.D. Brown¹, A. Kurkchubasche¹, W.B. Dobyns².* 1) Rhode Island Hosp, Brown University School of Medicine, Providence; 2) Department of Human Genetics, University of Chicago School of Medicine.

Two patients with severe microcephaly associated with similar MRI findings of smooth gyral pattern also had intestinal obstruction at the level of the jejunum. Case 1: Born at 35 weeks gestation with birth weight 2300 gms and head circumference 29 cm. Prenatal ultrasound showed microcephaly, echogenic bowel and hydronephrosis. Amniocentesis revealed a normal male karyotype. Physical examination showed a sloping forehead, poorly reactive pupils, thin, downturned lips, a small phallus and nonpalpable testes. He was found to have a small ASD and a large PDA, which was ligated. There was also right-sided UPJ obstruction and a small left kidney. BAER testing showed profound neurosensory deafness. At operation, he was found to have multiple jejunal atresias with a distal "apple-peel" deformity. All intestinal segments were salvaged with anastomoses over a stent. MRI showed severe microcephaly, relative frontal lobe hypoplasia, reduced number of gyri causing a simplified gyral pattern, and normal cortical thickness. He developed spasticity by 6 months and seizures by 14 months. Case 2: Born at 36 weeks gestation with birth weight 2055 gms and head circumference 24 cm. Prenatal ultrasound showed microcephaly and bowel that was echogenic and distended. The forehead was sloping, but there were no other dysmorphic features. Audiologic testing was abnormal. At laparotomy he was found to have two sequential type I jejunal atresias amenable to tapering jejunoplasty and repair. Cranial imaging studies were similar to case 1. Muscle tone at birth was normal, but spasticity gradually developed. The combination of marked microcephaly together with simplified cerebral gyral patterns (MSG) has been reported (Barkovich et al, 1998). Our two patients fit into this classification and in addition required surgery for jejunal obstructions. One of the reported patients with MSG also had jejunal atresia. We therefore suggest that this combination of anomalies constitutes a unique syndrome.

Hypoplastic corpus callosum and pachygyria in a patient with distal 2q duplication syndrome. *J. Ibrahim¹, B. Levy^{1,2}, T. Dunn¹, J. Willner^{1,2}, N. Kardon^{1,2}.* 1) Department Human Genetics, Mount Sinai School of Medicine, New York, NY; 2) Department of Pediatrics, Mount Sinai School of Medicine, New York, NY.

We describe an infant with a distinct set of clinical findings. A 3020g male infant was born at 39 weeks gestation to a 29 year old woman. At birth the infant was vigorous but became floppy and cyanotic at ten minutes of life. He was noted to have multiple dysmorphic features including small anterior fontanelle, short forehead, hypertelorism, down-slanting eyes, "cobblestone" appearance to the brow when furrowed, broad and prominent nasal bridge, anteverted nares, and increased folding of the superior helices. The chest was broad with low, wide-spaced nipples. The fingers were tapered and fifth digit clinodactyly was present bilaterally. The thumbs were proximally placed. The scrotum was hypoplastic and the testes were undescended. MRI of the brain revealed a hypoplastic corpus callosum and pachygyria.

G-banding revealed additional material at the chromosome 2 terminus. The origin of the additional material was determined to be chromosome 2 using FISH with a Whole Chromosome Paint. The revised karyotype was 46,XY inv dup(2)(qterq37.1).ish2(wcp2+). Further delineation of the breakpoints using CGH is anticipated.

Our patient exhibits typical features of the duplication 2q syndrome but this is the first detailed description of abnormal brain morphology. Several cases of duplication 2q exist in the literature but none are limited to the region q37.1@qter. This case further refines a critical region for the duplication 2q syndrome through the use of molecular cytogenetic techniques.

De novo chromosomal insertion in a child with autism. *L. Mehta¹, A. Yenamandra², R. Perrone², P. Koduru².* 1) Div Medical Genetics; 2) Dept. of Laboratories, North Shore University Hospital-N.Y.U School of Medicine, Manhasset, New York.

A 2.5 year old male was evaluated because of autistic features. He was delivered at full term following an uncomplicated pregnancy, to non-consanguineous parents with no family history of relevant problems. Parents were not exposed to radiation or chemicals. Motor development was mildly delayed. There were major communication and language delays with poor social interactions. Hyperactivity, poor eye contact, rocking, humming and repetitive hand movements were present. Criteria for a DSM-IV diagnosis of autism were met. There were no significant dysmorphisms or birth defects. Head circumference was 25-50%. Growth was normal. Cafe-au-lait pigmentation was present in the right axilla. Head CT scan, EEG, ophthalmology exam, plasma amino acids, urine organic acids and fragile X DNA analysis were normal. Peripheral blood chromosome analysis revealed additional material on chromosome 14q and an abnormal chromosome 18q. Fluorescence in situ hybridization with chromosome 14 and 18 painting probes (Vysis, Inc.) revealed an apparently balanced insertion of a chromosome 18 segment into chromosome 14. Therefore the karyotype was 46,XY,add(14)(q24),abn(18q).ish ins(14;18)(q24;q12.2q21.1). Parental chromosomes were normal. Reports of autosomal chromosome abnormalities in autism are infrequent but of potential interest in identifying candidate genes. Regions of interest have included chromosome 15q11-13, 7q21, 10q21.2, 16q23 and 17p11.2. Random unbalanced chromosome rearrangements presenting with autism, birth defects, dysmorphisms and mental retardation are also reported. Our patient presented with typical autistic features without physical abnormalities. No other etiology for his symptoms was found on testing. The apparently balanced karyotype therefore implicates sequences at 14q24, 18q12.2, 18q21.1 as candidate gene regions for autism. Detecting the chromosome abnormality has enabled accurate genetic counseling for this couple. Recurrence risks are lower than the 3-5% quoted for autism of multifactorial etiology. Normal prenatal karyotyping would be reassuring in future pregnancies.

Microcephaly with pachygyria: a new pedigree with autosomal recessive inheritance. *G.H. Mochida¹, L. Flores-Sarnat², A. Del Angel³, J.G. Gleeson⁴, C.A. Walsh¹.* 1) Neurology, Beth Israel Deaconess Medical Center, Boston, MA; 2) Neurology, Children's Hospital, University of Washington, Seattle, WA; 3) Genetics, Universidad Nacional Autónoma de Mexico, Mexico City; 4) Neurosciences, University of California, San Diego, CA.

Microcephaly with pachygyria is a heterogeneous group of brain malformations. We report 3 siblings with pachygyria and one healthy sibling, born to consanguineous Mexican parents. Patient 1 is a 12-year-old girl with severe developmental delay. She cannot walk or talk, has spastic quadriparesis and generalized tonic-clonic epilepsy controlled by valproic acid. Brain MRI showed a diffusely simplified convolutional pattern with broadening of gyri (pachygyria), paucity of posterior cerebral white matter and enlarged lateral ventricles. Patient 2 is a 7-year-old boy with microcephaly noted since birth. He started walking after age 3 years, and currently speaks several words. Patient 3 is a 2-year-old girl born at 32 weeks gestation. She can stand unsupported, but independent walking and speech have not yet developed. Brain MRIs of patients 2 and 3 were nearly identical in showing diffuse pachygyria and no ventricular enlargement. Patients 2 and 3 have not had seizures. All 3 patients had a normal karyotype. Though generally seen as sporadic cases, there are several reports of pachygyria with autosomal recessive (AR) inheritance. This family appears to be distinct from previously reported cases in clinical features, however. Sztriha et al. (1999) reported AR cases of "microcephaly with simplified gyral pattern", arthrogryposis and a poor neurological outcome. Peiffer et al. (1999) described 6 affected children, each with seizures beginning during the first several months of life. The clinical heterogeneity between families supports the hypothesis of multiple AR loci for pachygyria. The identification of genes responsible for this condition will facilitate a better understanding of genetic mechanisms of brain development, and will most likely lead to a revised classification of this entity in accordance with genetic etiologies. GHM is a Howard Hughes Medical Institute Physician Postdoctoral Fellow. Supported by the NINDS.

Secondary Leber Hereditary Optic Neuropathy (LHON) mitochondrial DNA mutations 4216 and 4917 associated with migraine-like episodes. *E.C. Lichtenberg¹, L.E. Walsh^{1,2}, J. Biller²*. 1) Dept Medical & Molec Genetics, Indiana Univ, Indianapolis, IN; 2) Dept Neurology, Indiana Univ, Indianapolis, IN.

We report the case of a 16-year-old girl with a 5-year history of headaches. Her headaches were accompanied by photopsias and vomiting. She complained also of fatigue, blurred vision, and paresthesias. Past medical and developmental histories were normal otherwise. Family history was notable for her mother's depression, anxiety disorder, and severe headaches; her father's apparent job-related hearing loss; her grandparents' strokes and hearing loss in senescence, and a maternal second cousin with complicated migraine and seizures. Physical examination was normal except for very mild left arm weakness. MRI scans of the brain showed mild cerebral atrophy, and scattered periventricular and subcortical hyperintensities localized mostly to parietal and occipital regions, but also involving the thalami, consistent with ischemic changes. Cerebral angiography was normal. Neuro-ophthalmologic evaluation revealed questionable visual field deficits, but no findings consistent with LHON. Ancillary testing (including CSF examination) for inherited and acquired causes of stroke was negative except for mitochondrial DNA testing. Tests for common MELAS mutations in peripheral blood and muscle were negative. However, analysis of muscle tissue for primary and secondary LHON mutations identified 2 mutations within the mt DNA ND1 and ND2 domains (4216, 4917). Muscle biopsy was normal otherwise and plasma lactate was 1.4 mmol/ml. The patient's clinical presentation suggested MELAS; however, her MRI lesions were atypical for this disorder. Familial hemiplegic migraine and CADASIL were also considered. The 4216 and 4917 mutations have been reported in controls and in optic neuropathy associated with primary LHON mtDNA mutations. The 4216 mutation also has been reported previously to be more frequent in young patients with stroke than in controls. These putative secondary mutations may represent coincidental findings in our patient, may modify her condition, or may be markers for disorders of mtDNA maintenance, suggesting either a nuclear defect or unknown primary mtDNA mutation.

Fits and pulmonary haemorrhage: Cortical malformation disorder and Ehlers-Danlos syndrome. S.

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Ehlers-Danlos syndrome (EDS) is a heterogeneous group of heritable connective tissue disorders, characterized by joint hypermobility, skin extensibility and tissue fragility. Cortical malformation disorders are due to a disturbance in neuronal migration during fetal development. They include lissencephaly (agyria/pachygyria spectrum), polymicrogyria and bilateral periventricular nodular heterotopia (BPNH).

Periventricular heterotopias and polymicrogyria have been described in association with features of EDS in 5 cases. We report the clinical, pathology and radiological findings of a woman who presented with haemoptysis who has both familial BPNH and features of EDS. This case provides further support for the association of a cortical malformation disorder with EDS. We will also consider the pathogenesis of pulmonary haemorrhage in the context of these 2 disorders.

Expanded phenotype of partial trisomy 11q. *H. Zhao, A. Rope, R.I. Blough, H.M. Saal, R.J. Hopkin.* Division of Human Genetics, Children Hospital Medical Center, Cincinnati, OH.

Three new patients with partial trisomy 11q are presented. Case 1 presented with seizures, metabolic acidosis, and hypoxia. He was found to have an unbalanced paternally derived trisomy 11 with partial monosomy 5q [46,XY,der(5)t(5;11)(q35.3;q23.3)pat]. He also had a complex congenital heart defect (tetralogy of Fallot with pulmonary atresia). Case 2 had lissencephaly, a malformed epiglottis, congenital heart defect (AV canal), hypoplastic kidneys with renal insufficiency, and cleft palate. Her karyotype was 46,XX,der(9)t(9;11)(p23;q21)mat. Case 3 presented with dysmorphic features, developmental delays, mental retardation, heart defect (atrial septal defect), seizures, and malformed epiglottis. Her karyotype was 46,XX,add(11)(q23).ish11(WCP11+). There were several features common to trisomy 11q identified in the three patients, including developmental disabilities, hypotonia, microcephaly, growth retardation, congenital heart defects, cleft palate, short nose, micrognathia, external ear anomalies, and renal anomalies. Of interest is the new finding of epiglottis malformation in cases 2 and 3. This leads to the speculation that there may be at least one gene located on 11q that plays a role in the development of the epiglottis.

Familial aggregation of trichotillomania in four living generations. *H.H. Wright¹, C.M. Wolpert², S.A. Ravan¹, R.K. Abramson¹, M.L. Cuccaro¹, M.A. Pericak-Vance².* 1) University of South Carolina, Columbia, SC; 2) Duke University Medical Center, Durham, NC.

Trichotillomania (TTM) is defined as an impulse-control disorder characterized by recurrent pulling on one's own hair (DSMIV; American Psychiatric Association, 1994). This classification is controversial as some researchers suggest that the disorder may be a subtype of obsessive compulsive disorder (OCD) or a feature of Tourette syndrome. The genetic basis of TTM is not well defined. Case reports of familial aggregation exist and there are several spontaneous-occurring animal models of compulsive self-grooming that are considered useful parallels for the study of TTM in humans (feather picking in birds, psychogenic alopecia in cats). We have ascertained a family with 7 individuals with TTM in 4 living generations suggesting apparent autosomal dominant transmission. Diagnosis and characterization of the phenotype in this family was performed using the TTM Diagnostic Interview, the National Institute of Mental Health (NIMH) TTM scales, and the Yale-Brown Obsessive-Compulsive Scale Symptoms Checklist (self-report version). All affected family members met the diagnostic criteria for TTM, but not for OCD or Tourette syndrome. These data warrant the further investigation of the underlying genetic basis of TTM as an independent disorder separate from OCD and Tourette syndrome.

Using the Aberrant Behavior Checklist (ABC) to Refine the Autistic Disorder (AD) Phenotype. *S.A. Ravan¹, M.L. Cuccaro¹, D.D. Wallace¹, R.K. Abramson¹, C.M. Wolpert², S.L. Donnelly², M.A. Pericak-Vance², H.H. Wright¹.* 1) Neuropsychiatry & Behavior, Univ South Carolina Sch of Med, Columbia, SC; 2) Center for Human Human Genetics, Duke University Medical Center, Durham, NC.

The ABC is a standardized measure of maladaptive behavior in individuals with developmental disorders (Aman & Singh, 1986). This 58-item rating scale yields composite scores in five areas that reflect mood and behavioral states. Recent studies using the ABC have reported specific profiles for developmental disorders such as Angelman syndrome (Clarke & Marston, 2000). Autistic Disorder (AD) is a neurodevelopmental disorder with core impairments in social, communicative, and behavioral functioning. Accurate phenotype description is crucial to genetic studies (Ashley-Koch et al. 1999; Cook et al. 1999; Pericak-Vance et al. 1999). Due to heterogeneity, AD phenotype refinement is ongoing.

As part of a genetic study of AD, caregivers of AD individuals (n=131) completed the ABC to define AD behaviors (Mean ABC factor scores Irritability=12.8, Lethargy=11.6, Stereotypy=6.4, Hyperactivity=17.7, and Inappropriate Speech=3.6). Principal component and factor analyses were run to examine factor structure of the ABC in an AD sample. A four-factor solution (promax rotation) yielded interpretable results. The basic factor structure was similar to that of the original ABC as well as Brown et al (1998). In our analysis the Irritability and Hyperactivity factors formed a single factor, Disruptive Behavior. The other factors (Lethargy, Stereotypy, and Inappropriate Speech) were confirmed. Mean scores for ABC factors were contrasted as a function of language level based on the Autism Diagnostic Interview-Revised (Lord et al 1994). Significant between group differences were found on the Inappropriate Speech factor.

This study is the first to examine the usefulness of the ABC for AD. The ABC may help examine other behavioral features of the AD phenotype in a quantitative fashion. This may prove a useful strategy to stratify individuals with AD in genetic studies.

Does imprinting influence brain development in X monosomy? *S.M. Zeng¹, M. Haberecht², S. Eliez², W.E. Brown², I. Warsofsky², J. Yankowitz¹, A.L. Reiss².* 1) Univ Iowa College of Med; 2) Stanford Univ School of Med.

Several studies have evaluated whether the phenotype of Turner syndrome (TS) is influenced by imprinting. We used the parental origin of the X chromosome as a surrogate for potential imprinting effects. We evaluated whether parental origin of the retained X affects brain development and structure. Blood was collected from 23 TS (45, X) pts, their parents, and sibs as available. DNA was extracted in standard fashion and genotype analysis used PCR amplification at 9 polymorphic sites on the X chromosome (HAR, HPRT, DXS6799, DXS6089, DXS8378, DXS9895, DXS9898, DXS101 and DXS1120). 3D-SPGR magnetic resonance images (TR=35, TE=6, FA=45, NEX=1, 256x192, FOV=24, 124 1.5 mm slices) were analyzed using BrainImage generating gray, white, and total lobar tissue volumes. Statistical analyses were performed on brain measurements using the Mann-Whitney U test.

Eight of 23 (34.7%) samples had a paternally derived X (Xp) and 15/23 (65.3%) a maternally derived X (Xm). Compared to controls, Xm subjects showed decreased gray matter volume (11%) in the right occipital lobe and white matter loss (12%) in the left and right occipital lobe. Also, increased gray matter volumes (11%) were observed in Xm subjects in the cerebellum bilaterally. Girls with Xp demonstrated decreased gray matter (7%) in the right temporal lobe relative to controls.

Our preliminary data suggest that girls with Xm show decreased lobar tissue volumes in key posterior brain regions compared to controls. This is consistent with previous studies suggesting parietal/occipital abnormalities as well as variable involvement of the cerebellum in TS. We also observed varying patterns of structural brain alterations on the basis of TS parental inheritance subgroup. These findings are interesting in light of reports showing that the Xp group have superior verbal and higher-order executive function skills. Future studies will investigate the differential contributions of X monosomy and parental origin to parieto-occipital structure, spatial cognition and executive function in TS.

X-linked cerebellar atrophy and mental retardation: a new syndrome. *P.L. Plotner¹, S.T. Iannaccone², J.K. Mills², S.H. Blanton³, H. Northrup¹.* 1) The University of Texas Houston Medical School, Houston, TX; 2) Texas Scottish Rite Hospital, Dallas, TX; 3) University of Virginia Health Science Center, Charlottesville, VA.

We report a family with a distinct phenotype of cerebellar atrophy and mental retardation that appears to be inherited in an X-linked recessive fashion. The family contains 13 individuals with 4 affected males in 2 generations: 3 second generation sons and 1 third generation grandson. The second generation is the product of non-consanguineous parents of Hispanic origin. The third generation is the product of a second generation daughter and her non-consanguineous spouse. A simulation study found the maximum obtainable LOD score to be 2.7.

All affected males have had early onset of mental delay leading to an eventual diagnosis of mental retardation (borderline to mild) and significant verbal delay. Cerebellar signs develop by mid-childhood, usually by age 11 years. These signs include: ataxia, tremor, decreased DTRs, and poor saccadic eye movement. No dysmorphic features are present.

Affected adult males display cerebellar atrophy by MRI. In contrast, MRI of one affected male child (age 5) did not reveal cerebellar atrophy. He has speech and intellectual delay but has not yet developed overt cerebellar signs. An affected adult male has slow nerve conduction velocity, a nerve biopsy suggestive of metabolic impairment, and a muscle biopsy demonstrating acute and chronic denervation. The family has been tested for SCA 6 and 7 with negative results. Routine neuropathy testing has been within normal limits. Linkage analysis was performed using 18 dinucleotide markers spaced approximately at 10cM intervals on the X chromosome: 15 markers do not show linkage, the other 3 markers are not informative.

We conclude that this family represents a newly described non-specific X-linked mental retardation syndrome associated with cerebellar atrophy. Additional testing of markers at 5cM intervals will be performed to define a candidate gene region.

A new variant for Autosomal Recessive Spinal Muscular Atrophy in Childhood. *L. Viollet¹, D. Leclair-Richard², P. Burlet¹, C. Belser¹, E. Vial¹, S. Bertrand¹, S. Lefebvre¹, A. Munnich¹.* 1) INSERM U-393, Hopital Necker, Paris, France; 2) Service de Pediatrie et Reeducation Neuro-respiratoire, Hopital Raymond Poincare, 92380 Garches, France.

Spinal muscular atrophy (SMA) is a group of neuromuscular disorders in childhood characterized by progressive anterior horn cell degeneration, leading to early or juvenile onset paralysis, amyotrophy and denervation. The genetic heterogeneity of autosomal recessive SMA in childhood has been demonstrated. Indeed, two disease loci have been hitherto identified on chromosomes 5q13 (proximal SMA) and 11q13-q21 (Distal SMA with Diaphragmatic Palsy), respectively. The most common form is the proximal SMA that results from mutations of the Survival Motor Neuron (SMN) gene. We described here a new variant of autosomal recessive SMA in a consanguineous family of four affected children. The three affected daughters presented with motor weakness at the age of 3 years. Proximal and distal muscle weakness progressively worsened, leading to the lost of walking ability at the age of 7,5 years. Walking disability was due to bilateral foot malposition and pelvic girdle weakness. Severe involvement of scapular muscles was also present, with hand weakness and fasciculations. Early hyperlordosis and scoliosis were noted. No sensory defect was detected and deep tendon reflexes were abolished. Electromyography revealed partial and progressive denervation, with normal motor and sensory nerve conduction velocity. The affected son showed a milder phenotype with similar proximal and distal distribution, that began at the age of 10 years. Linkage analyses in this family showed that two affected sibs were carrying discordant haplotypes at both the 5q13 and the 11q13 loci. These data support the view that this variant form of autosomal recessive SMA maps to a third locus. A genome wide search is under current investigations and should allow the mapping of a novel locus for autosomal recessive SMA.

Co-occurrence of Autistic Disorder and Chiari type 1 malformation in Five Patients: Further Evidence for Potential Cerebellar Involvement in AD. *C.M. Wolpert¹, D.S. Enterline¹, M.L. Cuccaro², S.L. Donnelly¹, S.A. Ravan², R.K Abramson², H.H. Wright², M.C. Speer¹, M.A. Pericak-Vance¹.* 1) Dept Medicine, Duke Univ Medical Ctr, Durham, NC; 2) W.S. Hall Psychiatric Institute, University of South Carolina, Columbia, SC.

Autistic Disorder (AD) is a neurodevelopmental disorder with a well established genetic component; the general population prevalence is 2-10 per 10,000. Chiari type 1 malformation (CM1) is a rare condition defined as ≥ 5 mm bilateral herniation of the cerebellar tonsils through the foramen magnum. The general population prevalence of CM1 is estimated to be 1/5000. Through two separate studies, one investigating the genetic basis of AD and one investigating the genetic basis of CM1, we have identified 5 patients in which AD and CM1 co-occur. For these cases, AD was confirmed using the Autism Diagnostic Interview-Revised and CM1 was diagnosed if there were ≥ 5 mm of bilateral descent of the cerebellar tonsils. Of 289 AD individuals, only 84 had neuroimaging studies of the brain. Of these 84 patients, three probands (families 7501, 7707, 7939) were found to have CM1. Interestingly, two of these three families also have a history for AD: the proband in family 7501 has a brother with AD; the proband in family 7707 has a half brother with AD. There is no family history of AD in family 7939. No other family members have had MRI studies. From the CM1 study, monozygotic twin males (family 9430) were concordant for CM1 and AD. The parents of these boys underwent MRI studies; the father was also found to have CM1. The mother's MRI was normal. Since AD and CM1 are rare disorders, this co-occurrence, although in a small series, suggests a common underlying mechanism for both disorders in a subset of cases. This finding is especially intriguing in light of research that indicates potential cerebellar involvement in AD. In addition, the presence of CM1 and AD in families suggests the possibility of an objective phenotype for stratifying AD and CM1 families, leading to the identification of genes important in the development of both conditions.

Late-onset central hypoventilation with hypothalamic dysfunction: a possible neural crest disorder. S.

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Congenital central hypoventilation syndrome (CCHS) is characterized by inadequate ventilation during sleep in the absence of primary neuromuscular, pulmonary or cardiac disease. Usually, it occurs as an isolated phenotype and in 16% of patients in association with Hirschsprung disease (HSCR). Current records indicate that there are 160-180 living children with CCHS and approximately 100 published case reports. Idiopathic central hypoventilation has occasionally been reported in previously healthy children after infancy [11 cases]. The late onset-CHS also features evidence of hypothalamic dysfunction, including hyperphagia, hypersomnolence, thermal dysregulation, emotional lability, and endocrinopathies. Both disorders occur in patients with a histologically normal central nervous system. While CCHS is known to be associated with mutations in at least 3 genes [RET, GDNF (glial cell line derived neurotrophic factor), and EDN3 (endothelin 3)], such associations with the late onset type are not known. Here, we report on a 6-year old hispanic boy with "Late Onset-CHS with endocrinopathies" who was normal until 2 years of age. His symptoms consisted of obesity, recurrent episodes of somnolence/lethargy, temperature instability, psychomotor delay, thoracic paraspinal ganglioneuroma and hypothalamic endocrinopathies. His VER and ERG responses were abnormal. An antibody screen was negative. Medroxyprogesterone did not significantly improve his response to mechanical ventilatory support. Direct mutation analysis of the RET, GDNF and EDN3 genes is in progress. Given the similarities between the congenital and late-onset forms of central hypoventilation syndromes, we propose that the 2 disorders may be etiologically related. Adequate mechanical ventilation in such patients is necessary to prevent sleep apnea, cardiac failure and hypoxic seizures.

Huntington's disease with childhood onset of mental retardation and possible late onset disease in the father. *J.G. Pappas, D. Punales-Morejon, V.B. Penchaszadeh.* Div Medical Genetics, Beth Israel Medical Ctr, New York, NY.

Huntington's disease (HD) is an autosomal dominant disorder with usual onset between the 35th and 40th year of life. The clinical manifestations include personality changes of a varied but non-specific kind, followed by choreiform movements and dementia. The inheritance of HD shows anticipation and complete penetrance. The molecular basis of this disease is expansion of the trinucleotide repeats in the Huntington's disease gene. Childhood onset of HD with behavior disorder, mental retardation, seizures and movement disorders has been documented. All reported childhood cases have documented typical HD in their fathers. 90% of the individuals with onset before 30 years of age have 55 or more repeats (normal range 10-35) (Illarioshkin et al., Ann Neurol 1994;36:630-635; Lucotte et al., Hum Genet 1995;95:231-232). Our case is a 44 year old black male from the Dominican Republic who presented with mental retardation, tremors of the extremities and inability to sit still for long. The onset of the progressive mental retardation was at 7 years of age and was diagnosed at his school. He followed special education for developmentally delayed children. He had no malformations or dysmorphic features and no family history of mental retardation. His mother age 65 years old reported that the tremors of our patient started at age 40 and they are worsening. DNA analysis of the HD gene revealed 49 CAG repeats. His father died by suicide at age 63 and reportedly had occasional extremity tremors and personality changes during the last year of his life; which suggests late onset HD. Our case suggests that HD can be a cause of childhood onset of mental retardation even in the low range of trinucleotide repeats associated with the disease. The prevalence of the disease is much higher in whites than blacks. The phenotype in black individuals of Dominican descent like our case may be associated with early onset of mental retardation.

Beckwith-Wiedemann syndrome (BWS)-related features and segmental monosomy 18q. V. Gaston¹, C. Houdayer¹, V. Soupre¹, J. Couturier², R. Couderc¹, C. Gicquel¹, A. Munnich³, M-P. Vazquez¹, Y. Le Bouc¹, M. Bahuau¹. 1) Explorations Fonctionnelles (V.G., C.G., Y.LB.), Biochimie (C.H., R.C., M.B.), Chirurgie Maxillofaciale (V.S., M-P.V., M.B.), Hôpital Trousseau, Paris, France; 2) Cytogénétique, Institut Curie, Paris, France; 3) Génétique Médicale, Hôpital Necker Enfants-Malades, Paris, France.

Beckwith-Wiedemann syndrome (BWS, MIM 130650) is a tumor-predisposing condition with cardinal features of exomphalos, macroglossia, and gigantism in the neonate. BWS and BWS-related tumor growth have been linked to genetic imbalance in the 11p15.5 region resulting in overexpression of *IGF2*, with downregulation of *H19*. Further adding to the genetic complexity of this disorder, point mutations of neighboring *p57(KIP2)/CDKN1C* tumor-suppressor may also cause WBS. It is now admitted that several genetic loci, 11p15.5-linked or not, may determine BWS or BWS-related features. In particular, deletion 18q22.1 was shown in an infant with findings of macroglossia, umbilical hernia, and neonatal hypoglycemia. Authors stated that this region of chromosome 18 might contain a transactivator for maintenance of *IGF2* imprinting since RT-PCR analysis showed biallelic expression of *IGF2*, uniparental disomy (UPD) ruled out. We report on a patient, term-born by C-section in a state of apparent death, who had findings of cleft palate, auditory canal stenosis, bilateral inguinal hernia, clubfoot, and pulmonic valve stenosis. Hypotonia was major and contact was poor. There was de novo translocation 46, XY, der(18)t(18;?)(q21;?), leading to segmental monosomy 18q and to trisomy for another chromosome. A small ring chromosome was seen in 2/12 mitoses. His tongue became progressively enlarged, causing major prognathism and hindering feeding. Adenoidomegaly caused airway to obstruct. Molecular analysis for *IGF2* showed absence of UPD in both lymphocyte and tongue (glossectomy-derived) DNAs, and normal, paternal-only, expression from tongue RNA. This observation confirms the existence of a genetic locus for WBS-related features on 18q. However, unlike previous findings, transactivation of *IGF2* through loss of imprinting does not seem to be the underlying mechanism.

Atypical Radiation-Sensitive Chromosome Breakage: New Syndromic Presentations. *S.E. Palmer¹, R.A. Gatti², B. Hirsch³, P.J. Concannon⁴, J.E. Anderson¹*. 1) Univ. Texas Health Sci. Cntr, San Antonio; 2) Univ. of California at Los Angeles; 3) Univ. of Minnesota, Minneapolis; 4) Virginia Mason Research Cntr, Seattle.

Clinical presentations of radiation-sensitive chromosome breakage disorders include Ataxia Telangiectasia and Nijmegen Breakage Syndrome (NBS). NBS displays microcephaly, short stature, and distinct facies that resemble Seckel Syndrome, due to a gene on 8q21. We present 2 families who display radiosensitivity by Colony Survival Assay, but do not fit key features of NBS. FAMILY 1: A 6 y.o. girl has cleft palate, ASD, no speech, pseudoobstruction, and distinct dysmorphic facies (broad face, small nose, ptosis, brachycephaly, unusual brow) that do not resemble Seckel Syndrome. Stature and head size are normal. She developed myelodysplasia. Routine chromosomes (blood, skin) were normal at multiple labs, but breakage studies showed increased spontaneous breakage, normal SCE and Mitomycin C studies, but borderline DEB results. She lacks the common Slavic NBS1 mutation; addition studies are pending. FAMILY 2: A 45 y.o. male presented with myelodysplasia. He has microcephaly, aged skin, short stature, developmental disability, cryptorchidism, and Seckle-like facies. He tolerated bone marrow transplantation without radiation. His 49 y.o. sister has identical exam, abnormal menses, and mild anemia. Their age is atypical for NBS, as the registry lists none older than 20s likely due to malignancy and immunodeficiency. Routine chromosomes (blood, marrow) were normal at multiple labs, but breakage studies showed mild increase in spontaneous breakage, normal SCE and MMC, and borderline DEB results. NBS1 gene analysis failed to reveal a mutation; Western blotting for nibrin showed normal levels. These findings suggest 1) routine chromosome analysis may be inadequate to detect increased spontaneous breakage; 2) myelodysplasia with any syndromic feature should prompt radiosensitivity studies; 3) adult females with radiosensitive breakage raise debates about mammography in homozygotes at high risk for malignancy; 4) these 2 families may represent new syndromes and loci encoding radiation-sensitive chromosome breakage.

Retinal Capillary Hemangioma and von Hippel-Lindau Disease: Phenotypic Variability and Progression. *A.D. Singh¹, M. Nouri², N. Perez¹, C.L. Shields¹, J.A. Shields¹*. 1) Oncology Service, Wills Eye Hospital, Philadelphia, PA; 2) Department of Ophthalmology, Hahnemann University, Philadelphia, PA.

Introduction: von Hippel-Lindau (vHL) disease is a multisystem disorder with a predilection for the central nervous system (CNS) and retina. It is of particular relevance to the ophthalmologist because retinal capillary hemangioma (RCH) is the most frequent and often the earliest manifestation of vHL disease. The inheritance of vHL disease is autosomal dominant with high penetrance. The penetrance of vHL disease is age dependent achieving full penetrance by 65 years of age. The cumulative probability of developing RCH increases progressively with age reaching the maximum of 70% by the age of 30 years. Purpose: To study the phenotypic variability and progression of RCH in patients with or without vHL disease. Methods: Retrospective case series of 68 patients with RCH (median follow up: 77 months). Results: 31 (46%) patients had vHL disease (family history: 19, multiple RCH: 9, systemic features 3). The average age at diagnosis was 24 years. 51 (75%) of patients had unilateral involvement and the 25% were bilaterally affected. Of 170 tumors, location was juxtapapillary in 18%, superotemporal periphery in 44%, and other quadrants in 38%. Only 3 of 50 eyes with solitary RCH developed additional tumors and all three cases had vHL disease. Conclusions: RCH can vary in number, location, and size. However, isolated RCH and RCH associated with vHL disease are clinically indistinguishable. Additional tumors do not develop in the majority (94%) of cases presenting with solitary RCH. Whereas solitary RCH can occur sporadically or in association with vHL disease, multiple RCH is highly suggestive of vHL disease.

Familial recurrence of congenital heart defect in patients with d-transposition of the great arteries. *M.c. Digilio¹, B. Marino¹, A. Toscano¹, S. Anaclerio¹, A. Giannotti¹, V. Colloridi², R. Calabrò³, M.G. Russo³, M. Marasini⁴, E. Banaudi⁵, B. Dallapiccola².* 1) Medical Genetics and Cardiology, Bambino Gesù Hospital, Rome, Italy; 2) Cardiology and Genetics La Sapienza University, Rome Italy; 3) Cardiology Monaldi Hospital, Napoli Italy; 4) Cardiology G. Gaslini, Genova Italy; 5) Cardiology Regina Margherita Hospital, Torino Italy.

Transposition of the great arteries (TGA) is considered to be rarely associated with genetic syndromes and to have a low risk of recurrence among relatives of affected patients. We investigated the overall occurrence of congenital heart defect (CHD) and the recurrence risk in relatives of 370 patients with d-TGA evaluated from January 1997 to April 2000 in five Italian pediatric institutions. All probands had atrial situs solitus, d-ventricular loop with patent atrioventricular valves and TGA. Family pedigrees were constructed to include first- and second-degree relatives and first cousins. Results: One or more relatives affected by CHD were found in 40/370 (10.8%) families. D-TGA was present in 7 relatives, l-TGA (congenitally corrected) in 5, discordant CHD in 31. The occurrence of CHD in sibs was 8/433 (1.8%), in parents 4/740 (0.5%), in first cousins 15/3284 (0.5%), in uncles/aunts 3/2120 (0.1%), in grandparents 1/1474 (0.06%). In 11 cases CHD was diagnosed in more distant relatives. Conclusions: 1) Our results show that d-TGA is not always a sporadic occurrence in the families; 2) In our series the recurrence risk rate for sibs is 1.8%; 3) In some families with high recurrence of d-TGA and/or l-TGA a mendelian mechanism of inheritance can be hypothesized; 4) It is noticeable that d-TGA and l-TGA (congenitally corrected) can segregate in the same family due to at present surprising and unknown genetic mechanisms.

Progressive heart block linked to SCN5A, and long QT syndrome with bradycardia linked to HERG: ion channel disorders with bradycardia in two Scottish families. *J.C. Dean¹, J. Watson¹, K. Jennings², M. Cowie², N. Haites¹.* 1) Dept Medical Genetics, Medical School, Aberdeen, Scotland, UK; 2) Dept Cardiology, Aberdeen Royal Infirmary, Aberdeen, Scotland, UK.

Long QT syndrome may be associated with mutation in any of six genes encoding components of either potassium or sodium ion channels. Recently, mutations in one of these genes, SCN5A, have also been found in Brugada syndrome (paroxysmal VT without long QT) and in two families with hereditary cardiac conduction defects (Lenegre-Lev disease). In one of these families, the conduction defect was progressive and associated with right bundle branch block, while in the other the atrio-ventricular block was non-progressive. We report a third family with inherited heart block, in which the ECG demonstrates normal QRS complexes, but progressive prolongation of the PR interval from the early twenties, resulting in complete heart block by the forties. While some relatives in earlier generations died suddenly, younger affected individuals have been treated by pacemaker insertion. Molecular studies using markers D3S1298 and D3S1100, flanking the SCN5A gene, are consistent with linkage to this locus, with a maximum LOD score of 1.04 at zero recombination. Neither of the two previously reported mutations have been detected in this family: a search for a new mutation is under way. In another family, who have long QT syndrome, bradycardia following beta-blockade was associated with episodes of ventricular tachycardia. The arrhythmias were abolished by permanent pacemaker insertion. Molecular studies in this family using D7S505, D7S636 and D7S483 suggest linkage to HERG (maximum LOD score 1.81 at zero recombination). This is in keeping with other studies suggesting that HERG associated long QT families require pacing more frequently than other genetic forms of long QT syndrome. These families with symptomatic bradycardia of differing origins contribute to increasing knowledge of genotype-phenotype correlations in the cardiac ion channelopathies.

Initial clinical manifestations of Arrhythmogenic right ventricular cardiomyopathy (ARVC). *E. Dicks¹, K. Hodgkinson¹, S. Connors¹, M. Norman^{2,3}, L. Thierfelder², M. Longley², W. McKenna³, A. Bassett⁴, P. Parfrey¹.* 1) Faculty of Medicine, Health Sciences Centre, St. John's, NF, Canada; 2) Max-Delbrück Centre, Berlin, Germany; 3) St. George's Hospital, London, UK; 4) University of Toronto, Ontario, Canada.

Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is a difficult to diagnose AD disorder with genetic and clinical heterogeneity and variable expressivity. To determine the natural history of ARVC5 (OMIM) we investigated six Newfoundland families linked to 3p23 comprising 235 family members who were either affected clinically, obligate carriers, or at 50% risk of inheriting the gene. Of 174 (74%) subjects born after 1929, 87(50%) were considered affected either by i) a high risk DNA haplotype (n=43) or ii) obligate carrier (n=6), or iii) sudden death or defibrillator or pacemaker or defibrillation before 50 years of age (n=38); 37 (22%) were considered unaffected (low risk DNA haplotype); with the remaining 50 (29%) unknown: (29 (59%) of these have DNA testing in progress). Medical record data on the first cardiac manifestation was available for 124 subjects. Symptoms of arrhythmia (palpitation, syncope and sudden death) were assessed. 50 of 52 affected males had a clinical problem: syncope in 11 (22%) palpitations in 10 (20%) and sudden death in 22 (44%). Clinical presentation differed in 21 of 35 affected females: syncope in 3 (14%) palpitations in 15 (71%) and no sudden deaths. Mean age of onset was 30 years (95% CI 28-33), and 39 years (CI 33-45) for males and females respectively (rr=2.3, p=0.003). In unaffected subjects, a cardiac symptom was recorded in 6 of 16 males (38%) and 9 of 21 females (48%) with mean age of onset 36 years (CI 29-43) and 38 years (CI 28-49) respectively. The positive predictive value (ppv) for syncope or palpitations in diagnosing ARVC in subjects who did not present with sudden cardiac death was 84% in males with 70% sensitivity and 72% ppv in females with 60% sensitivity. We conclude that ARVC5 expresses in early adulthood, frequently with sudden death in males. Expression is later and less malignant in females. Diagnostic utility of symptoms of arrhythmia in females is poor.

Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) in six Newfoundland families: a sex-limited disease with malignant phenotype in males. *K. Hodgkinson¹, E. Dicks¹, S. Connors¹, L. Thierfelder², M. Norman^{2,3}, W.*

McKenna³, M. Longley², A. Bassett⁴, P. Parfrey¹. 1) Faculty of Medicine, Health Science Ctr, St John's, NF., Canada; 2) Max-Delbruck Centre, Berlin, Germany; 3) St. George's Hospital, London, UK; 4) University of Toronto, Ont., Canada.

Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is a difficult to diagnose autosomal dominant disorder with variable expressivity. Six large (up to 8 generations) Newfoundland families are linked to chromosome 3p23 (ARVC5: OMIM), suggesting a common founder. Comprehensive clinical and family history information was obtained in 235 subjects (135 males, 100 females); data were less likely to be available for subjects born in earlier generations. Subjects were either affected or at 50% pedigree risk; linkage data were available for a subset. Disease status was defined as: i) affected (high risk DNA haplotype, OR obligate carrier, OR sudden death under 50 years, OR cardiac intervention for ARVC with defibrillator/pacemaker or defibrillation under 50 years) n=118 (50%), ii) unaffected (low risk DNA haplotype), n=37 (15%), or iii) unknown, n=80 (35%). Genotyping results pending on DNA obtained from 34 subjects in the unknown group will raise ascertained disease status to 189 (80%) of the sample. 58 of 74 affected males, have died; 42 sudden deaths (72%). Mean age at death was 41 y (95% CI: 38-44); 22% died by age 30 y, 51% by 40 y, 82% by 50 y, and 92% by 60 y. 10 of 44 affected females have died; 2 sudden deaths (20%). Mean age at death for females was significantly older: 66 y (CI: 60-72); there were no deaths under 40 y, 15% by age 50 y, and 28% by 60 y. Relative risk comparing deaths in males to females was 6.1 (p=0.00001). Results were not significantly different when subjects born after 1929 were analysed separately; mean age at death for affected males was 37y (CI: 34-41). The results indicate a clear sex-limitation for ARVC5 with males having a malignant phenotype expressed in early-mid adulthood.

Familial occurrence of both left and right heart hypoplasia in two different sibships. *J. Martinovic¹, D. Bonnet², A. Benachi³, L. Fermont², F. Encha-Razavi¹, M. Vekemans¹.* 1) Service d'Histo-Embryologie et de Cytogenetique, Hopital Necker, Paris, France; 2) Service de Cardiologie Pediatrique, Hopital Necker, Paris, France; 3) Service de Gynecologie et Obstetrique, Hopital Necker, Paris, France.

A familial recurrence is very well known in isolated congenital hypoplastic left heart syndrome (HLHS). Familial occurrence of hypoplastic right heart syndrome (HRHS) has also been reported. Here we report on a familial occurrence of left and right heart hypoplasia in one pair of dizygotic twins as well as in two sibs from a second sibship where one sib had a hypoplastic left heart and the other sib had a right heart hypoplasia. In the first family, a 23-week male fetus presented isolated HLHS with a small left ventricular cavity, mitral stenosis and aortic atresia. His twin sister presented a HRHS with a small right ventricular cavity, pulmonary atresia, intact ventricular septum and ostium secundum atrial septal defect. No other anomalies were found in both fetuses. In particular, a single DCDA placenta without anastomoses between both circulations was found. Chromosome analysis was normal and family history was unremarkable. In the second family, a male infant was born with prenatally diagnosed isolated classic HRHS. Family history revealed the presence of HLHS in a first cousin female infant. According to the developmental-mechanistic theory both HLHS and HRHS belong to the group of flow lesions. A high concordant recurrence rate is usually observed in families with classic HLHS, affected members having a left-sided blood-flow lesions. To our knowledge this is the first report on the occurrence of the left and right heart hypoplasia among the sibs of the same family. Therefore, the present observation is puzzling and may be useful in stimulating further studies and shed light on the etiology of these congenital heart diseases.

Complex cardiac anomalies in deletion 9p22, duplication 5q34 of maternal origin: a case report. *F. Lacbawan¹, S. Schonberg², D. Walton¹, P. Koroulakis¹, H. Thackray³*. 1) Children's National Medical Center, Washington, DC; 2) American Medical Laboratories, Chantilly, VA; 3) Medical Genetics Branch, NHGRI, NIH, Bethesda, MD.

We report a deletion of 9p22-pter with duplication of 5q34-qter in an infant with multiple congenital anomalies, most notably complex cardiac defects. The infant was born at 36 weeks gestation, and after eight years of infertility and two previously documented spontaneous abortions, to a mother with balanced translocation t(5;9)(q34;p22). There was in utero growth retardation and oligohydramnios. Emergent cesarean delivery was performed for fetal distress. Echocardiogram revealed ASD, VSD, bicuspid aortic valve, abnormal pulmonary arch, coarctation of the aorta, inadequate left ventricular volume, and bidirectional shunting through the ductus arteriosus. Head ultrasound was normal and renal ultrasound revealed echogenic kidneys. She was hypotonic, and microcephalic with bifrontal narrowing. She had a flat sloping forehead, neutral palpebral fissures, small nose with tall and broad nasal bridge and anteverted nares, and posteriorly rotated, low set ears with overfolded helices. There was cleft palate, micrognathia, short broad neck, and widely-spaced nipples in a broad flat chest. She had single palmar creases, broad thumbs, and bulbous digits with hyperconvex, hypoplastic nails. The infant succumbed upon withdrawal of life support in her second week of life.

The clinical findings of the deletion 9p syndrome (9p del) have been well described. The deletion in most cases is de novo and the most common breakpoint is at 9p22, as in our patient. Our patient had similar clinical characteristics of 9p del, however, the typical findings of upslanting palpebral fissures, long philtrum, hypoplastic supraorbital ridges and hernias were not readily observed. In contrast, 9p del is not usually associated with such severe cardiac defects. We believe this patient's manifestations expand the clinical picture associated with deletion 9p and duplication 5q.

CONGENITAL ANOMALIES ASSOCIATED WITH FETAL CONGENITAL HEART DISEASES. M.

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The objective of the study was to evaluate the major extra cardiac malformations associated with congenital heart defects (CHD) of the fetus. Out of 2,454 fetuses with CHD coming from 709.030 fetuses registered by 20 European registries of congenital anomalies, 761 (31,0%), of them had associated malformations including 375 (49,3%) chromosomal abnormalities, 104 (13,0%) non chromosomal syndromes and 282 (37,0%) multiply malformed fetus with non chromosomal and non other recognized syndromes. Among the chromosomal abnormalities 239 were Down syndromes, 59 trisomies 13 and 14 Turner syndrome. The extra cardiac malformation were central, renal, digestive, musculo-skeletal anomalies and facial clefts. Twenty eight of the 104 recognised non chromosomal syndromes were deletion 22q11, 17 had situs anomaly and 15 VATER association. The major extra cardiac anomalies of non syndromic multiply malformed were renal, digestive including abdominal wall defects, central nervous system and musculo-skeletal. This study shows that it is very important to have a complete investigation of the fetus after the ultrasonographic detection of a fetal CHD as 31% of the fetuses have cardiac malformations. The detection of these associated anomalies will improve the prenatal diagnosis and therefore the care of the patient.

Tetralogy of Fallot and dysmorphic features associated with partial trisomy 8q/monosomy 1q. A. Yenamandra¹, S. Buckwald², R. Schiff², N. Cagungan¹, L. Trinchitella¹, P. Koduru¹, L. Mehta². 1) Dept. of Laboratories; 2) Dept. of Pediatrics, North Shore University Hospital-N.Y.U School of Medicine, Manhasset, New York.

Tetralogy of Fallot (TOF) and conotruncal heart defects are heart defects best known to be associated with chromosome 22q11.2 microdeletion. Few other consistent chromosome abnormalities are reported with this defect. The SLV rec(8) chromosome is highly associated with conotruncal heart defects. We report an infant with partial trisomy 8, monosomy 1q and TOF. A full term male infant was delivered to a 33 year old G2P1 mother. Birth weight was 2685 gm and head circumference was 34.5 cm. There was early onset of seizures, poor respiratory effort and low oxygen saturations. Cardiology evaluation diagnosed tetralogy of Fallot, valvar and infundibular pulmonic stenosis, right aortic arch and patent foramen ovale. Facial dysmorphisms consisted of downslanting palpebrae, broad nasal root, prominent nasal tip, bilateral pre-auricular pits and unfolded ear helices; toes were overlapping with a deep groove between digits 1 and 2. There was a right undescended testis. Renal ultrasound showed mild hydronephrosis. Brain MRI, chemistries and metabolic screen were normal. Chromosome analysis was 46,XY,der(1)t(1;8)(q44;q21.2). Fluorescence in situ hybridization with the diGeorge syndrome critical region probe (Vysis, Inc) was normal. Maternal chromosome analysis was normal. The father carried a balanced reciprocal translocation between chromosomes 1 and 8. The infant inherited his derivative 1 chromosome after adjacent-1 segregation, and was therefore trisomic for segments 8q21.1-ter and monosomic for segments 1q44-ter. He had overlapping features of trisomy 8q and 1q monosomy. Congenital heart defects and seizures are reported in 1q44 monosomy. Ear anomalies and pits, bulbous nose and plantar grooves are reported in partial trisomy 8. Tetralogy of Fallot is highly associated with the SLV rec(8) chromosome[inv(8)p23q22] but is not a feature of 8q duplication, mosaic trisomy 8 or deletion 8q. Hence, this region of chromosome 8 influences cardiac development by unknown mechanisms.

Does every Neonate with Down syndrome need an Echocardiogram? *V. Shashi, M.N Berry, W. Covitz.* Pediatrics, Wake Forest Univ Sch Med, Winston-Salem, NC.

Echocardiography is the accepted method of screening for congenital heart defects (CHD) in neonates with Down syndrome, since a large number can be missed on physical examination (PE) and early intervention can minimize consequences such as pulmonary hypertension. Surprisingly, few studies have evaluated the efficacy of an electrocardiogram (ECG) along with PE, in detecting CHD. We performed a retrospective chart review of 287 children with Down syndrome. Exclusion criteria were: prenatal echo; echo performed prior to PE; age over 1 month at exam; insufficient information; PE by individuals other than a geneticist/cardiologist. With these exclusions, 49 medical records were analyzed, from 1991 onwards. The age ranged from 1 day to 1 month. CHD was detected in 33 of 49 neonates (67%). This high percentage is likely to be due to ascertainment bias. Of the 33 with proven CHD, 13 (39%) had a normal cardiac exam and thus would have been missed on PE alone. Of these 13, 10 defects were hemodynamically significant (AV canal, VSD, secundum ASD). Three were not hemodynamically significant (small muscular VSD, small ASD). In the 10 significant CHD cases, the ECG was abnormal and indicative of the underlying abnormality in all the 6 cases in which it was available. No ECG was available in the other 4 significant defects. In all 3 cases with insignificant defects, the ECG was normal. The sensitivity of PE alone was 61%; and the specificity was 90%;. The ECG was highly predictive of CHD, with a specificity of 98%;. In instances where the PE was erroneously normal, the ECG correctly identified cases with a significant CHD (6/6, no ECG available in 4). Although we speculate that the ECG may be predictive in the other 4 cases, for the purpose of this analysis, it is assumed that the ECG in those 4 cases was not abnormal. Thus, the sensitivity of ECG in combination with PE is 78%. Interestingly, in all 3 cases with hemodynamically insignificant CHD, the ECG was normal. Our conclusions are: 1) PE alone is insufficient to detect CHD in neonates with Down syndrome. 2) A combination of PE and ECG will detect the majority of significant CHD. This may be an alternative approach when an echocardiogram is not readily available.

Trisomy 18 syndrome with unusual karyotype. *C.M. Cazano, S. Chowdhury, A.F. Grieg, M.J. Macera, S.M. Kleyman, R.S. Verma.* Departments of Pediatrics and Medicine, Wyckoff Heights Medical Center, Brooklyn New York Hospital/Weill Medical College of Cornell University, New York, N.Y.

Trisomy for the entire chromosome 18 and monosomy for a large portion of the long arm [18q-] or short arm [18p-] exhibit typical features resulting in at least three types of clinical syndromes that can be recognized even prior to cytogenetic evaluation. A new born boy delivered after 29 weeks of gestation with birth weight of 875 gm, was referred for cytogenetic evaluation to rule out trisomy 18 syndrome. The mother is a 39 years old G3P2002, and the father is 50 years old. Prenatal care was provided. Maternal triple marker screening was negative. Amniocentesis was offered but she refused. Major clinical features of the proband include: marked hypotonia, muscle waste, skin with poor fat content, triangular face, very small palpebral openings, box forehead, prominent occiput, small sternum, separation of wide nipples, hepatomegaly, small pelvis, clenched hands and rocker-bottom feet. Chest X-ray and EKG showed abnormal right superior axis. Echocardiogram showed dextrocardia, pulmonic valve dysplasia, PDA and severe lung dysfunction with evidence of pulmonary hypertension. He had hematuria at birth. GI studies showed an esophageal pouch. On the fourth day, the child developed sepsis. On the fifth day, he had severe hemolysis, hyperbilirubinemia, anemia, metabolic acidosis, and despite exchange transfusion he expired on the sixth day with severe NEC, shock and respiratory failure. Cytogenetic findings with GTG and FISH-techniques revealed an abnormal 47,XY,t(3;18)(q23;q11.2)mat, +18.ish(wcp3+,wcp18+;wcp3+,wcp18+) karyotype. The mother was a carrier of the balanced translocation i.e. 46,XX,t(3;18)q23;q11.2). The father was cytogenetically normal. The proband inherited the balanced translocation plus a normal chromosome 18, resulting in trisomic condition due to 3:1 segregation at meiosis in the mother that was confirmed by centromeric alphoid heteromorphism. To the best of our knowledge, such cytogenetic findings have not been previously reported.

Preliminary Analysis of Recurrence Risk and Familial Aggregation in Lipo(myelo)meningocele. *C. Drake¹, T. George¹, E. Melvin¹, J. Nye², A. Franklin¹, D. Enterline¹, S. Guy², M. Speer¹, NTD Collaborative Group³.* 1) Medicine, Duke University Medical Center, Durham, NC; 2) Pediatrics, Northwestern University Medical School Chicago, IL; 3) NTD Collaborative Group.

Neural tube defects (NTDs) are a common, complex birth defect, caused by genetic and environmental factors with two main classifications: open and closed. Open NTDs occur when the brain and/or spinal cord is exposed at birth through a defect in the skull or vertebrae. Closed NTDs have a layer of skin covering the spinal defect. Common examples of closed NTDs are lipo(myelo)meningocele and lipomeningocele, in which fatty elements infiltrate into the meninges and/or spinal cord.

Given the similarities in the bony defects of the open and closed NTDs, the two may result from the same genetic mechanisms. To investigate this hypothesis, we calculated recurrence risk for NTDs when a proband has lipo(myelo)meningocele. We also identified aggregation in extended pedigrees. We ascertained 59 Caucasian families in which the proband had lipo(myelo)meningocele. A standardized three generation pedigree was obtained.

In the 23 younger siblings of the probands, there were no recurrences of NTDs although there were precurrences. Four pedigrees indicated familial aggregation. One family had an affected sibling with anencephaly and another family had an affected maternal half sibling with lipo(myelo)meningocele. Two families each had two additional affected relatives. Family 8642 had a sibling with spina bifida and a paternal half sibling with myelocystocele. Family 8776 had a sibling and a maternal first cousin with spina bifida. The sample is currently being expanded. Familial aggregation maybe the result of environmental factors or pure chance and does not prove genetic involvement. However, these families are consistent with a genetic component in lipo(myelo)meningocele and suggest that lipo(myelo)meningocele may be genetically related to the open forms of NTD.

Premature Ovarian Failure and Marfanoid Features: A new association? *O. Goker-Alpan¹, C. Kozma², L.M.*

Nelson³, C.A. Francomano⁴. 1) NICHD/HDB, National Institutes of Health, Bethesda, MD; 2) Department of Genetics, Georgetown University Medical Center, Washington DC; 3) NICHD/DEB, National Institutes of Health, Bethesda, MD; 4) NHGRI/MGB, National Institutes of Health, Bethesda, MD.

Premature ovarian failure (POF) is defined as cessation of ovarian function under the age 40 and affects one percent of women of reproductive age in general population. POF is characterized by secondary amenorrhea, hypoestrogenism and elevated gonadotropin levels. We describe for the first time a group of patients with POF and Marfanoid features. Four patients (ages 28-36) were found to have POF as evidenced by elevated gonadotropins. The age of onset of POF ranged from 26 to 34 years. Karyotyping from peripheral blood lymphocytes and skin fibroblasts failed to show any chromosomal abnormalities. Fragile X premutation carrier state was ruled out by molecular and cytogenetic studies. One patient was evaluated for Marfan syndrome prior to her referral. All patients had a history of myopia, joint laxity, joint pains and one patient suffered from recurrent dislocations as a teenager. Easy bruising and fragility of skin were reported in three patients. There was no history of Marfan syndrome in patients' families. High arched palate, reduced upper to lower segment ratio, increased arm span to height ratio and subjective arachnodactyly were among the significant findings in all four patients. Three had pes planus with medial displacement of medial malleoli and genu recurvata. One patient had scoliosis. None of them had aortic dilatation on echocardiography. Their ophthalmologic evaluation did not show ectopia lentis, and none met the diagnostic criteria for Marfan syndrome. Ovarian dysfunction is not a feature in any of the known connective tissue disorders. These findings may represent an unrecognized connective tissue abnormality associated with POF. The mechanism of our observed association between premature ovarian failure and Marfanoid features remains to be determined.

Bilateral anterior temporal lobe cystic leukoencephalopathy with microcephaly, minor dysmorphisms, and psychomotor retardation. *G.M. Bibat¹, E.R. Melhem², S. Naidu¹*. 1) Div Neurology & Dev Medicine, Kennedy Krieger Institute/Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Neuroradiology, Johns Hopkins Hospital, Baltimore, MD.

We report 3 unrelated patients (2 boys and 1 girl) aged 20 months to 7 years with cystic leukoencephalopathy, born to healthy non-consanguineous parents. Severe cognitive and motor impairment with spasticity occurred within the first year of life. They were microcephalic with minor dysmorphisms but seizure free and without retinal abnormalities. Cytogenetic and metabolic (including mitochondrial, lysosomal and peroxisomal) screening in all three patients were unremarkable. However, MRI of the brain consistently revealed multiple foci of abnormal white matter, and bilateral cystic changes in anterior temporal lobes. The constellation of clinical and neuroimaging findings form a unique diagnostic entity. There are some MRI similarities to vacuolating leukoencephalopathy described by Van der Knaap, M et.al. (Ann Neurol 37:324-334, 1995) and Singhal, B et.al. (Ped Neurol 14:291-296, 1996) mapped to chromosome 22qter (Topcu, M et.al. Am J Hum Genet 66:733-739, 2000). However, these patients differ from our cases in having megalencephaly, absence of dysmorphisms and a milder cognitive impairment. Also, their degree of white matter abnormality is more severe than in our patients and their survival into adulthood is associated with progressive severe spasticity. Three Turkish cases reported by Olivier, M et.al. (Neuroped 29:225-228, 1998) may resemble our patients except that all our cases had microcephaly with mild dysmorphic features and were seizure free. Our cases are of European descent suggestive of multiethnic prevalence of this entity. Despite our patients being singletons, its occurrence in Turkish sibs of consanguineous parents suggests an autosomal recessive mode of inheritance.

Deletion of chromosome 14q32.2 is apparently associated with scoliosis and retrognathia. A. Jain^{1,2}, A. Krauss¹, L. Lumicao¹, M.L Alonso², M.J Macera³, R.S Verma^{2,3}. 1) Pediatrics, Jamaica Hosp. Medical Center, Jamaica, N.Y; 2) The New York Hospital/Weill Medical College of Cornell University New York; 3) Wyckoff Heights Medical, Brooklyn, N.Y.

Based on the clinical findings, a single band deletion of a chromosome has been associated with a specific clinical syndrome. The results of an exhaustive literature search, in which overlapping features associated with deletion of this chromosomal band have been compared, has with a few exceptions, produced no definite conclusion [Brewer et al Am J Hum Genet 63:1153-1159,1998]. We report a case of a new born baby girl who is the product of a 37 weeks gestation delivered by caesarian section. She has minimal dysmorphic features, with a birth weight of 1450 gm. A head sonogram was normal with a circumference of 30 cm. She had an episode of seizures and was placed on phenobarbitol. The most significant findings were scoliosis and retrognathia. To the best of our knowledge, a single band deletion of 14q of this minute nature has never been reported earlier. The initial cytogenetic diagnosis using routine GTG banding was 46,XX,del(14)(q32.2). This was revised to 46,XX,del(14)(q32.2).ish del(14)(q32.1q32.3)(14q Tel Vysion x2) after a 14q telomere probe(Vysis) was applied. Since telomeric signals were observed over both homologous, the deletion of only band q32.2 was concluded. It is tempting to hypothesize that these two abnormalities may be related with band q32.2 on the long arm of chromosome 14. However, scoliosis has been associated with 2p15-13, 6q13, 15q12 in earlier cases. Furthermore, it is our belief that earlier cases need to be reevaluated as they can easily be misidentified as terminal deletions rather than interstitial.

Sanjad-Sakati Syndrome (MIM 241410) in a West-African family: The search for an ancestral mutation. M.

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Syndromic hypoparathyroidism is rare and includes such conditions as the Kenny-Caffey Syndrome (KCS) and the Sanjad-Sakati Syndrome (SSS). SSS was first described by Sanjad in 1988 as an autosomal disorder characterized by growth retardation, congenital hypoparathyroidism, dysmorphic facies, seizures and retardation. Patients lack the bony changes seen in KCS. A recessive form of KCS (MIM 244460) was demonstrated in 1997 and has recently been shown to be allelic with SSS. All of the patients reported to date have originated from the Arabian peninsula. We have identified sibs from the West-African nation of Gambia.

Case Report: **Case 1** was the 1680 gm product of a 37 week gestation delivered by SVD to a 26 year old Gambian mother who is the first cousin of the father. At birth, the HC was 28.5 cm and the length was 43 cm. The forehead sloped, the metopic suture was prominent and the eyes and ears were large. He developed hypocalcemic seizures at 7 months of age. The parathyroid hormone level was undetectable and he is profoundly retarded. **Case 2** is the 6 year old sister of the proband. She was the 1765 gm product of a term pregnancy complicated by IUGR and oligohydramnios delivered by section because of a breech presentation. The H.C. at birth was 27.5 cm and the length was 42.5 cm. The anterior fontanelle was closed, the forehead sloped, the eyes and ears prominent and she had bilateral branched simian creases. She developed congestive failure secondary to a fenestrated VSD which resolved spontaneously and she too is profoundly retarded. At 5 years of age her Ca^{++} was 8.2 (ionized 4.4), and parathyroid hormone 13.

The gene for SSS has been localized to 1q42-q43. SSS and KCS are now felt to not only be allelic but caused by the same ancestral chromosome in all cases studied thus far. Our family suggests the possibility of genetic heterogeneity or the migration of the ancestral chromosome to West Africa and is the first case in an African family.

Clinical overlap between Trichorhinophalangeal and Floating Harbor Syndromes. *J.W. Innis^{1,2}, J.L. Schuette¹, D.M. Martin¹*. 1) Department of Pediatrics, The University of Michigan, Ann Arbor, MI; 2) Department of Human Genetics, The University of Michigan, Ann Arbor, MI.

Trichorhinophalangeal (TRP) and Floating Harbor syndromes are multiple congenital anomaly syndromes with involvement of craniofacial and skeletal structures. Trichorhinophalangeal syndrome exists as three autosomal dominant forms (I, II, III) and one autosomal recessive form, with variable clinical features such as short stature, mild facial dysmorphisms, sparse hair, speech delay, and genitourinary abnormalities. Floating Harbor syndrome, also an autosomal dominant condition, was named after the two hospitals where it was originally identified and is characterized by short stature, expressive language delay, characteristic facies, and delayed bone age. TRP I is associated with mutations in TRPS1, a zinc finger transcription factor on 8q24. We report here a child with features that overlap significantly between TRP I and Floating Harbor syndromes. A.W. presented at age 2 months for evaluation of growth delay and multiple congenital anomalies. He was later found to have severe short stature, triangular face, short, light blonde hair, a prominent nose, protruding, low-set ears, a submucous cleft palate, ivory and cone epiphyses of the phalanges, Perthes disease of the left hip, and severely delayed expressive speech with normal speech reception abilities. Peripheral blood karyotype and FISH22q11deletion studies, endocrine evaluation (IGF-I, IGF-BP3) and hearing tests were normal. Family history revealed no similarly affected family members. Our patient's findings are more consistent with Floating Harbor syndrome; however, his features overlap significantly with TRP syndrome, emphasizing the importance of considering both diagnoses during evaluation of short stature, speech delay, and characteristic triangular facies. Molecular testing for TRPS1 mutations is pending.

Characterization of Asthma, Atopy and Associated Phenotypes in Families Identified Through Child Probands with Physician Diagnosed Asthma. *P.J Helms¹, W. Anderson², K. Barnes¹, A. Berg¹, S. Brewster³, K. Carlsen¹, J. Gerritsen¹, C. Haynes⁴, W. Lenney¹, P. Manasco², A. Roses², M. Pericak-Vance⁴, M. Silverman¹, P. Sly¹, M. Speer⁴, J. Sundy¹, J. Tsanakas¹, J. Vance⁴, M. Whyte¹, Genetics of Asthma International Network¹.* 1) Genetics of Asthma International Network; 2) Glaxo-Wellcome Inc., Research Triangle Park, NC; 3) Glaxo-Wellcome Research and Development, Greenford, UK; 4) Duke University Medical Center, Durham, NC.

Asthma is a common, complex disorder with both genetic and environmental components. To investigate the genetic basis of asthma, we have established an international network of 11 sites, each collecting 100 nuclear families with at least two siblings (age 7-35) with a physician diagnosis of asthma (PDA). **Patients and methods:** In all subjects (including parents) the following measurements were performed: respiratory questionnaire including detailed family history and environmental risk factor assessment; bronchial reactivity (PC20-methacholine); reversibility of airway obstruction; skin-prick tests and serum total IgE. **Results:** To date, 501 families (2089 individuals) have been fully ascertained. Of the affected children, 54% are boys with a median age of 12 and a median age of physician diagnosis of asthma of 4 yrs. Among asthmatic children eligible for methacholine challenge, approximately 52% demonstrated bronchial hyperreactivity (PC20 for FEV1 < 8mg/ml) and 75% had at least one positive skin prick test (> 2 mm greater than negative control). Approximately 7% of asthmatic children had PC20 = 0.12 mg/ml, representing one subset with more severe disease. 35% of the parents had asthma and the majority (74%) were skin prick positive to at least one allergen. In 63% of families, one or both parents reported a history of regular cigarette smoking. Blood samples have been collected for DNA and an approximately 7 cM resolution genomic screen initiated in the first 379 families. **Conclusion:** These data should provide insight into genetic factors and traits involved in asthma and other related phenotypes.

Molecular Basis of Bernard-Soulier syndrome. *D. Ng*¹, *S. Schonberg*^{2,3}, *N. Luban*³, *S. Kaler*⁴. 1) NICHD, National Institutes of Health, Bethesda, MD; 2) American Medical Laboratory, Chantilly, VA; 3) Children's National Medical Center, Washington, DC; 4) NINDS, National Institutes of Health, Bethesda, MD.

The platelet membrane glycoprotein (GP)Ib-V-IX complex is the receptor for von Willebrand factor and is composed of four membrane-spanning polypeptides: GPIb alpha, GPIb beta, GPIX, and GPV. A qualitative or quantitative deficiency in this complex causes the human platelet disorder Bernard-Soulier syndrome (BSS). BSS is an autosomal recessive disorder presenting with mild thrombocytopenia, circulating "giant" platelets and a bleeding phenotype. The bleeding in patients with BSS is disproportionately more severe than suggested by the reduced platelet count and is explained by a defect in primary hemostasis owing to the absence of the platelet glycoprotein (GP) Ib-V-IX membrane receptor.

We report on an infant with facial dysmorphism, a congenital heart defect and neonatal thrombocytopenia in whom heterozygous deletion of the chromosome 22q11 critical region consistent with velocardiofacial (VCF) syndrome was demonstrated by FISH analysis. The patient's father, who has a history of mild thrombocytopenia, did not harbor the 22q11 deletion. Maternal testing for platelet antibodies was negative, ruling out neonatal alloimmune thrombocytopenia (NAT).

Platelet surface glycoprotein studies in the infant demonstrated extremely reduced expression of GPIb beta, the gene for which localizes to 22q11. We speculate that this patient harbors a mutation in his paternally inherited GPIb beta allele; molecular studies formally evaluating this hypothesis are in progress in our lab. A literature search (6/00) indicates that mutations in GPIb beta in VCF patients with comparable hematological phenotype have been documented in only two individuals to date.

We wish to bring further attention to this association and suggest that (1) molecular testing for VCF be considered for thrombocytopenic infants when NAT has been excluded, and (2) 22q11 deletion VCF patients with enlarged platelets and bleeding tendencies be screened for alterations in GPIb beta.

Repetitive behaviors differentiate autism subgroups. *J.H. Miles, N. Takahashi, J.D. Mudrick.* Univ of Missouri, Columbia, MO.

Repetitive or stereotypic patterns of behavior and activities are one of three diagnostic criteria for Autistic Disorders (AD). We studied repetitive behaviors (RBs) in 153 individuals diagnosed with idiopathic infantile autism to develop a classification system, severity scale and protocol for study of the significance of RBs in our previously defined clinical subgroups. We question whether RBs in autism are a response, possibly palliative, to noxious external stimuli or an indicator of non-specific brain damage. Probandes were ranked, based on parent reports in a semi-structured interview, on four areas of RBs; hand flapping, spinning, humming and "other". The low RB category (46/153 - 30%) included probands with RBs in 0 or 1 of the areas and the high RB category (69/153 - 45%) group in 3 or 4. The high and low categories were compared for hypersensitivity to noise, resistance to change, and other variables. The high RB individuals were more likely to be hypersensitive to noise (59.4% vs 42.1%, $p < 0.025$) and resistant to change (84.0% vs. 57.9% $p < 0.001$). This supports previous findings that RBs in autism are a response to distressing sensations or situations (Hutt, 1968). The high and low RB groups did not differ significantly in physical morphology, head circumference, type of onset or family history of autism or psychiatric disorders. Thus RBs, by this grouping, did not help refine our previously defined autism subgroups (Miles, 1996). However, children with no prominent RBs were consistently different from those with prominent RBs: with a lower sex ratio (0.7:1 vs. 7.3:1, $p < 0.001$), higher IQs ($> 55 = 42\%$ vs 66% $p < 0.1$), higher SES ($p < 0.001$), less sibling recurrence risk for autism or autistic traits (0% vs 22% $p < 0.1$), and less family history of autism (0% vs 22% $p < 0.1$), affective disorders (17% vs 42% $p < 0.1$) or addictive disorders (17% vs 41% $p < 0.1$). Only the family history of obsessive compulsive disorder was higher in the no RB than the RB group (8% vs 6.7%). This suggests that children with no prominent RBs, though they fit DSM -IV AD criteria on the basis of interests or rituals may comprise a subgroup of AD more closely related to obsessive compulsive disorder.

Sternal cleft with supraumbilical midline raphe and hemangiomas: An X-linked lethal syndrome? *S.G. Kaler^{1,3}, H. Thackray²*. 1) Clinical Neurosciences Program, NINDS/NIH, Bethesda, MD; 2) Medical Genetics Branch, NHGRI, NIH, Bethesda, MD; 3) Children's National Medical Center, Washington, DC.

The association of sternal cleft (SC) and supraumbilical raphe (SR) is a midline developmental defect considered to occur sporadically. Embryologic formation of the thoracic and vascular structures involved in these defects suggests a critical period at approximately 8 weeks gestation. Review of the available medical literature indicates that this association has an equal sex predilection (42 examples reported). In contrast however, when SC and SR are accompanied by unilateral hemangiomas, a marked female predilection is observed (29 of 31 reported cases, greater than 93%). We report on a female infant with upper sternal cleft, midline abdominal raphe, and capillary and cavernous hemangiomas of the face, chest, left arm and left leg. The sternal cleft was covered with a thin translucent membrane; normal cardiac function and a small atrial septal defect were noted on echocardiogram. The abdominal raphe extended from the lower border of the sternal cleft to the umbilicus, with a small palpable midline muscular defect. Head, chest, and abdominal ultrasound exams excluded internal vascular malformations, and chromosomes were 46,XX. Repair of the sternal defect was performed without complications. During the first 7 months of life, the congenital hemangiomas progressed and then began a slow regression. At 10 months of age, cognitive and motor development were slightly delayed. The family history was notable for one prior miscarriage in the mother, and two female relatives with cutaneous hemangiomas (patient's maternal aunt and the maternal aunt's daughter). To our knowledge, a single gene etiology has not been considered for the constellation of SC with SR and hemangiomas. We speculate that this syndrome may represent an X-linked trait that is lethal in males, explaining the pronounced female predilection. X inactivation studies in our patient's peripheral white blood cells and cultured fibroblasts to assess for skewed inactivation will enable us to formally evaluate this hypothesis.

Molecular characterization of a 45,X/46,XY/46,X,r(Y) in a newborn male. *H.O. Shah¹, M.J. Macera², J. Sherman¹, B. Feldman¹, J.H. Lin¹, M. Pugliese¹, M. Chester¹, S.M. Kleyman², R.S. Verma².* 1) Nassau County Medical Center, East Meadow, NY; 2) Wyckoff Heights Medical Center, Brooklyn, NY.

Chromosomal mosaicism with 45,X/46,XY may be found in male patients with gonadal dysgenesis. Patients with such mosaicism can have a cell line with an additional marker chromosome. The incidence of cells with Y chromosome-derived markers must be investigated, because its presence predisposes these individuals toward gonadoblastoma formation. We were recently referred a baby boy who was the product of a full term uncomplicated pregnancy carried by a 23-year-old mother. Upon physical examination, the child had 3° hypospadias with chordee. The phallus length was 4.2 cm with good erectile tissue volume. The right testicle was descended and of normal size and consistency. The left testicle was not palpable and the left hemi-scrotum appeared uninhabited. Ultrasound evaluation revealed structures compatible with the upper 1/3 of a vagina. No testicle was identified on the left. Laboratory evaluations (including progesterone, DHEA, testosterone, LH and FSH) done at 3 days and 30 days of life were reasonably within normal limits for a male child. Initial cytogenetic evaluation with routine GTG-banding revealed a 45,X/46,XY/46,X,r(?) karyotype. QFQ-banding confirmed the presence of a Y chromosome. Molecular characterization with probes specific for the Y satellite III and the SRY gene (cepY, LSI SRY, Vysis) using the FISH technique on metaphase chromosomes confirmed the ring chromosome as a ring Y. The SRY gene and satellite III DNA were present on both the Y and r(Y) chromosomes. The breakpoints of the ring were determined to be r(Y)(p11.2q12). Approximately half of the cells containing the SRY locus were ring chromosomes. Analysis of the interphase cells (cepX, cepY, Vysis) gave the following information: 45,X.nuc ish Xcen(DXZ1x1)Ycen(DYZ1-)[140]/46,XY/46,X,r(Y).nuc ish Ycen(DZY1x1)[60]. It was determined that 70% of the cells were 45,X, 15% were 46,XY and 15% were 46,X,r(Y)(p11.2q12). With 30% of this child's cells containing a Y or r(Y) chromosome, it is imperative to follow his progress closely to prevent the formation of gonadal tumors.

A syndrome of autosomal dominant progressive renal failure and hypertension is mapped to chromosome 1q21-q22. *M. Shohat¹, T. Shohat¹, M. Yahav¹, G. Rechavi², T. Ilan², L. King³, D. Cohn³.* 1) Medical Genetics, Rabin Medical Ctr, Petah Tikva, Sackler School of Medicine, Tel Aviv University, Israel; 2) Pediatric Hematology and Oncology, Sheba Medical Center, Tel Hashomer, Sackler School of Medicine, Tel Aviv University, Israel; 3) Human Genetics and Ahmanson Department of Pediatrics, Cedars-Sinai Research Institute, UCLA, Los Angeles, CA, USA.

Several distinct clinical phenotypes have been described that lead to autosomal dominantly inherited end-stage renal failure. Examples include Alport syndrome and non-Alport nephritis, nephronophthisis/ medullary cystic kidney disease, interstitial nephritis, adult polycystic kidney disease, and various inherited glomerulopathies. Genetic loci for many of these conditions have been defined, and the disease genes have been isolated for several disorders in this group. However, in many of the aforementioned disorders there are additional, non-renal manifestations such as deafness or ocular abnormalities. In forms of nephropathy and hypertension without extrarenal findings (e.g. OMIM #161900), neither a locus nor a specific gene defect has yet been defined. We have ascertained a large Israeli family of Iraqi Jewish origin with an autosomal dominant form of adult onset nephropathy and hypertension. Hypertension was the presenting symptom in all of the patients. There was progressive renal failure and patients eventually required hemodialysis followed by renal transplantation. The aims of this study were to describe the clinical features in the family and determine the chromosomal location of the defective gene by a genome-wide linkage search. By performing a genome-wide linkage search, the disease gene was localized to chromosome 1q21; the highest lod score was obtained for the marker at locus D1S305, which yielded a maximum lod score of 4.71 at a recombination fraction of zero. Recombination mapping defined an interval of about 3 cM, between the markers at loci D1S2696 and D1S2635, which contains the disease gene. Localization of the disease-causing gene in this family represents a necessary step toward isolating the defective gene and providing a deeper understanding of the mechanisms of hypertension and progressive renal failure.

Severe neonatal hypotonia : high prevalence of Prader-Willi syndrome, myotonic dystrophy and spinal muscular atrophy in a molecular prospective study. *L. Van Maldergem, P. Hilbert, Y. Gillerot.* Centre de Genetique Humaine, Inst Pathology & Genetics, Loverval, Belgium.

Background Severe neonatal hypotonia in the absence of obstetrical complications remains a clinical problem with respect to its aetiology, and particularly to genetic disorders underlying the condition. Among these, Prader-Willi syndrome, the neonatal form of myotonic dystrophy and spinal muscular atrophy have recently become amenable to molecular diagnosis. **Methods** We performed DNA analyses in order to diagnose these three genetic disorders in 80 subsequent blood samples received for severe neonatal hypotonia between 1995 and 2000. **Results** 11 newborns (10 percent) were diagnosed with one of these disorders : six cases with Prader-Willi syndrome, four with Myotonic Dystrophy and one with Spinal Muscular Atrophy. **Conclusion** Prader-Willi, Myotonic Dystrophy and Spinal Muscular Atrophy altogether represent a significant proportion (13.75 %) of severe unexplained hypotonia of the newborn. It proves useful to perform a panel of three DNA tests in this situation in order to diagnose these conditions. It may prevent further invasive diagnostic procedures like muscle biopsy and allows early handling and genetic counselling for these disorders.

Johnson-McMillin syndrome, a neuroectodermal syndrome with conductive hearing loss and microtia: report of a new case. *D.N. Schweitzer¹, S. Yano², D.L. Earl¹, J.M. Graham, Jr.¹*. 1) Med Genet Birth Defects Center, Cedars-Sinai Medical Center, UCLA School of Medicine, LA, CA; 2) Childrens Hospital Los Angeles, LA, CA.

In 1983, Johnson et al. described 16 related individuals with alopecia, anosmia or hyposmia, conductive hearing loss, microtia and/or atresia of the external auditory canal, and hypogonadotropic hypogonadism inherited in an autosomal dominant pattern and with variable expressivity. Variable manifestations included mild facial asymmetry, mental retardation, congenital heart defect, and cleft palate. We describe an unrelated female patient with features resembling the family described by Johnson et al.. The proband was the 1950 gm product of a 38 week gestation born to a 19-year-old G1P0-1 woman and her healthy unrelated 21-year-old partner. Family history is significant for delayed menarche, slightly small auricles, possible hyposmia, and unilateral choanal stenosis in the mother. C-section at 38 weeks was done for oligohydramnios with severe IUGR. She presented with growth deficiency, alopecia, bilateral microtia with canal atresia, tetralogy of Fallot, posterior cleft palate, left choanal stenosis and right thumb polydactyly. Developmental evaluation at chronological age 13 months showed skills at 7 months. At 19 months length and weight were 6 SD below expected mean for age and OFC was 8 SD below mean for age. Total alopecia, brachycephaly, left facial palsy and hypotonia were noted. Phenotypic abnormalities involving neuroectoderm, craniofacial structures, heart and the endocrine system can be explained on the basis of involvement of the ectoderm and neuroectoderm of the first and second brachial arches, Rathkes pouch, and the diencephalon. Microtia with conductive hearing loss differentiates this condition from the other ectodermal dysplasias. Phenotypic expression appears to be quite wide, and the mother of our patient could be a mildly affected female who passed the condition to her daughter in an autosomal dominant fashion.

Cerebral vasculopathies as the cause of acute neurologic complications in Down Syndrome.

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There is a growing body of information that cerebrovascular disorders, particularly Moya Moya disease, may produce acute neurologic findings in Down Syndrome. Moya Moya is a chronic occlusive disorder of progressive stenosis of the supraclinoid internal carotid artery with secondary development of enlarged basal collateral vessels. We present an initial case of Down Syndrome with angiographically demonstrated localized internal carotid artery disease bilaterally with occlusion at the internal carotid origin on the right and a string sign beginning at the internal carotid origin on the left with collateral intracranial circulation. These findings are compared to Moya Moya. The patient is 3 y.o., WM, classic Down with trisomy 21, but no history of heart disease. Weakness of left arm was the presenting finding and within three weeks, patient lost movement of right arm and lost the ability to sit, crawl or pull to stand. Speech was unaffected. He was hospitalized two weeks later. MRI of cervical spine was normal; however, MRI of brain showed areas of subacute infarction. The mechanism of increased incidence of cerebrovascular abnormalities in Down Syndrome may be explained in several ways. Superoxide dismutase 1, interferon gamma receptor, and cystathionine synthase are proteins encoded on chromosome 21 which may increase arterial narrowing. The alpha chain of collagen type VI is also on chromosome 21, and previous post mortem studies have shown internal vascular thickening with vascular deposition. Excessive vascular thickening could be related to overexpression of collagen type VI in trisomy 21. An inflammatory or autoimmune mechanism could also be relevant to the pathogenesis. Obviously, further studies are needed. An unexplained propensity to cerebral artery abnormalities likely accounts for both our case and Moya Moya. We recommend consideration of cerebrovascular abnormalities in children with Down Syndrome who present with acute neurologic symptoms as well as appropriate neuroimaging studies.

Noonan-like/multiple giant cell lesion syndrome: a separate entity from Noonan syndrome? *D. Bertola¹, C. Kim¹, A. Pereira², G. Mota², J. Krieger², I. Vieira³, M. Valente⁴, M. Loreto⁵, R. Magalhaes⁵, C. Gonzalez¹*. 1) Dept. of Pediatrics, Instituto da Crianca - USP, Sao Paulo, Brazil; 2) Laboratory of Genetic and Molecular Cardiology, InCor, Sao Paulo, Brazil; 3) Dept. of Pathology, USP, Sao Paulo, Brazil; 4) Dept. of Radiology, Instituto da Crianca - USP, Sao Paulo, Brazil; 5) Dept. of Head and Neck Surgery, USP, Sao Paulo, Brazil.

Noonan syndrome (NS) is an autosomal dominant condition comprising short stature, craniofacial dysmorphisms, mainly hypertelorism, ptosis and downslanting of the palpebral fissures, webbed neck, congenital heart defects, cryptorchidism in males, skeletal defects and bleeding diathesis. A gene for NS has been mapped to the long arm of chromosome 12 (12q24), but some families affected by this disorder showed no linkage to this region, indicative of genetic heterogeneity. In 1974, Cohen et al. described a female patient presenting short stature, craniofacial dysmorphisms, short webbed neck, pulmonic stenosis, multiple lentigines and giant cell lesions of both bone and soft tissue. Cohen and Gorlin (1991) proposed that this disorder would represent a separate entity from NS and LEOPARD syndrome, although there was an evident clinical overlap among them. They named it Noonan-like/multiple giant cell lesion syndrome. We report on a family (a mother and two children) that has typical characteristics of NS and multiple giant cell lesions in maxilla and mandible. The linkage analysis performed in them showed that the three affected members studied share an identical 12q haplotype. We discuss the obvious clinical overlap between NS and Noonan-like/multiple giant cell lesion syndrome and we give further clinical and molecular support that these two entities could be allelic conditions.

A bovine model of a connective tissue disorder. *E. Felix*¹, *G. Sherman*³, *C. Gwilt*², *J. Winterboer*², *S. Beiraghi*^{1,2}, *D.J. Steffen*³, *M. Godfrey*². 1) Pediatric Dentistry; 2) Center for Human Molecular Genetics Univ. Nebraska Medical Center, Omaha, NE; 3) Veterinary Diagnostic Center, Univ. Nebraska, Lincoln, NE.

An animal model of a human disease has obvious advantages including the ability to study pathogenesis and novel therapies aimed at human disease. We have recently begun to study a family of Angus cattle with an apparent connective tissue disease. Some of the early pathology seemed to indicate the possibility of a Marfan syndrome like phenotype. The Marfan syndrome is an autosomal dominant heritable disorder of connective tissue with manifestations in the skeletal, ocular, and cardiovascular systems. Marfan syndrome is known to be caused by defects in the FBN1 gene encoding fibrillin-1. Most people with the Marfan syndrome have abnormal expression of fibrillin-1 in skin. Immunofluorescence studies of skin from affected and unaffected calves using a monoclonal antibody to fibrillin demonstrated normal staining in all animals. A possible Stickler syndrome like phenotype was suggested by their lax joints and unusual gait. Biochemical analysis of cartilage collagens was normal. As the calves aged and attempted to be more mobile, leg fractures became common and continued to point to a connective tissue etiology, possibly osteogenesis imperfecta. Studies to examine type I collagen are now underway. The fact that we have DNA on some 20 affected and unaffected calves, their dams, and their obvious germline mosaic sire makes this an informative pedigree for genetic analyses. While a specific diagnosis for these calves is currently unknown, the pleiotropic phenotype is reminiscent of human heritable connective tissue disorders.

A syndrome of microtia, cleft palate and Diamond-Blackfan anemia in first cousins. *K.W. Gripp¹, D. McDonald-McGinn¹, D. La Rossa¹, S.E. McKenzie², J. Lipton³, E.H. Zackai¹*. 1) Human Genetics, CHOP, Philadelphia, PA; 2) duPont Hospital, Wilmington, DE; 3) Schneider Children's Hospital, New Hyde Park, NY.

Diamond-Blackfan anemia (DBA) is a rare form of pure red cell aplasia presenting in infancy. Hematologic findings often include macrocytosis, and elevated hemoglobin F and erythrocyte adenosine deaminase(eADA) levels. Associated physical findings were noted in over 40% of cases; cleft lip or palate occur in 3%.

We report first cousins with microtia, cleft palate and hematologic abnormalities consistent with DBA. Pt 1 had bilateral microtia, micrognathia and a submucous cleft palate. Treacher Collins syndrome was suspected, but no TCOF1 mutation was found. Steroid responsive anemia developed at 16 months; macrocytosis and elevated hemoglobin F were consistent with DBA. Pt 2 had bilateral microtia, micrognathia, cleft palate, and mildly downslanting palpebral fissures. He had macrocytosis, elevated eADA and hemoglobin F, consistent with DBA. Our patients share bilateral microtia, midfacial hypoplasia, cleft palate and hematologic abnormalities of DBA. Except for a female with microtia, micrognathia and cleft palate (Hasan & Inoue, 1993), no cases of microtia and DBA have been reported. While the inheritance pattern in our cases could be X-linked recessive, this is an unlikely explanation for the female. Autosomal dominant inheritance with incomplete penetrance of DBA was confirmed by heterozygous mutations in the ribosomal protein 19 (RPS19) gene. Currently 46 cases with RPS19 mutations have been reported, none had microtia or cleft palate (Willig et al, 1999; Matsson et al., 1999). DBA is heterogeneous, only about 25% have identifiable RPS19 mutations. A second locus maps to 8p, and another locus is inferred. Sequence analysis of the RPS19 coding region did not identify a mutation in our cases, and 2 alleles were present in each. Segregation analysis is being performed. Once additional DBA genes are identified it will become clear if there is a correlation between the microtia-cleft palate phenotype and a specific genotype.

Two patients with distinct structural anomalies of chromosome 10 determined by advanced molecular cytogenetic techniques. *K.M. Dent¹, Z. Chen², B. Issa², A. Meloni-Ehrig², R. Shepard², D. Forsyth², X. Zhu², K. Carroll³, X. Guan⁴, A. Brothman², J. Carey¹.* 1) Medical Genetics/Pediatrics, Univ Utah, Salt Lake City, UT; 2) Cytogenetics Lab/Pediatrics, Univ Utah, Salt Lake City, UT; 3) Orthopedics, Univ Utah, Salt Lake City, UT; 4) National Center for Human Genome Research, Bethesda, MD.

Patient 1 is a 9-month-old female with multiple congenital anomalies and a mosaic female karyotype with an extra ring chromosome consisting of the entire short arm of chromosome 10 (10p). Spectral karyotyping (SKY), chromosome microdissection, and fluorescence in situ hybridization (FISH) clearly identified the mosaic extra ring to be completely of 10p origin. She had characteristics of children reported previously with trisomy 10p including growth retardation, developmental delay, hypotonia, congenital heart defect, and hypertelorism. In addition, she had severe joint laxity and a congenital knee dislocation, features not described previously. While trisomy 10p is a well-delineated chromosome abnormality, complete trisomy 10p has been reported only infrequently. Furthermore, this is the first reported case of isolated, complete trisomy 10p resulting from an extra ring chromosome. Patient 2 is a 4-month old male with a duplication of the proximal part of the long arm of chromosome 10 (10q11.23-10q23.2). This duplication was confirmed to be of chromosome 10 origin using FISH analysis with the chromosome 10 painting probe. The phenotypic findings are similar to those described in the three other published cases with the same karyotype and include growth retardation, prominent philtrum, bowed mouth, micrognathia, thick cupped ear helices, single crease on the fifth finger, and mild developmental delay. He also had a ventriculoseptal defect and atrial septal defect. Congenital heart defects have not previously been reported in patients with the same size duplication. Parental chromosomes were normal in both cases.

Our studies in these two patients further demonstrate the value of advanced molecular cytogenetic techniques in the identification of unknown structural rearrangements.

Clinical indications for Uniparental Disomy (UPD) testing in growth retarded patients: presentation of own results by searching for UPDs2, 6, 7, 14 and 20. *S. Mergenthaler¹, H.A. Wollmann², P. Kloos¹, B. Albrecht³, S. Spranger⁴, K. Eggermann¹, K. Zerres¹, T. Eggermann¹.* 1) Inst Hum Genet, Technical Univ , Aachen, Germany; 2) Childrens Hospital, Univ Tuebingen, Germany; 3) Inst Hum Genet, Univ Essen, Germany; 4) Inst Hum Genet, Univ Bremen, Germany.

The association of UPD and short stature has been established for different chromosomes and in several conditions. Therefore, we investigated a cohort of 21 patients with intrauterine and postnatal growth retardation for UPD of chromosomes 2, 6, 7, 14 and 20. Additionally, a healthy female carrier of two isochromosomes 2p and 2q and a newborn suffering from neonatal diabetes mellitus (NDM) were tested for UPD2 and 6, respectively. Typing of microsatellite markers revealed maternal segmental UPD14 and maternal UPD20 in one case each. The growth retarded newborn with segmental UPD14 showed some additional clinical signs common with the putative "maternal UPD14 syndrome". The maternal UPD20 patient showed minor features. Testing of the isochromosomes carrier revealed paternal UPD2p and maternal UPD2q. The woman was healthy and of normal growth. In the growth retarded neonate with NDM paternal isodisomy 6 was detected. Based on these results and those from the literature, the following guidelines for UPD testing can be delineated: maternal UPD2 and paternal UPD2p do not seem to be correlated with growth retardation. Paternal UPD6 seems to be restricted to growth retarded patients with NDM, maternal UPD7 to Silver-Russell syndrome patients. Testing for UPD14 is useful in growth retarded patients with maternal UPD14 syndrome features (hypotonia, developmental delay, small hands, hyperextensible joints, premature puberty). Whether maternal UPD20 is a frequent finding and associated with characteristic features remains to be elucidated by screening a larger number of growth retarded patients. Nevertheless, the phenotypic transitions in UPD patients might be fluid, therefore making consideration of different UPDs difficult, particularly among newborns with IUGR and unspecific dysmorphisms. This problem is increased by the possible finding of segmental UPD, thereby leading to greater phenotypic variability.

Co-localization of distichiasis-lymphedema-cleft palate (DLC) with distichiasis-lymphedema syndrome in

16q24.3. *M. Bahuau¹, V. Soupre¹, B. Karcenty¹, Y. Le Bouc¹, R. Couderc¹, A. Munnich², M-P. Vazquez¹, C. Houdayer¹.* 1) Biochimie (M.B., R.C., C.H.), Chirurgie Maxillofaciale (M.B., V.S., B.K., M-P.V.), Explorations Fonctionnelles (Y.LB.), Hôp. Trousseau, Paris, France; 2) Génétique Médicale, Hôp. Necker Enfants-Malades, Paris, France.

In view of identifying pedigrees with monogenic cleft lip and/or cleft palate, we investigated a family showing autosomal dominant segregation of upper- and lower-eyelid distichiasis (double row of eyelashes) in seven affected relatives over three generations, in addition to below-knee lymphedema of pubertal onset (lymphoedema praecox) in three of them. Two children had cleft palate/velum in addition to distichiasis, and without the previously reported association to the Pierre Robin anomalad. Other ophthalmologic anomalies included divergent strabismus and early-onset myopia. This family appeared to feature a discrete Mendelian condition distinct from previously delineated entities such as distichiasis (MIM 126300), type-1 (Nonne-Milroy's; MIM 153100), or type-2 (Meige's; MIM 153200) hereditary lymphedema. The clinical picture presented by this family also seemed to differ from distichiasis-lymphedema syndrome (MIM 153400), a disease entity recently mapped to 16q24.3, in that pterygium colli, congenital heart disease, or facial dysmorphism were not features here. Analysis using fluorescently-labeled microsatellite markers D5S498, D5S1353, D5S1354, D5S408, and D5S2006, excluded linkage to the type-1 hereditary lymphedema locus in 5q35.3. However, analysis using markers D16S511, D16S422, D16S402, D16S3037, D16S520, and D16S3074, mapped in 16q24.3, favored linkage with a maximum two-point LOD score of 2.10 at $q = 0$ for D16S3074. No recombination was observed between the markers studied. Neither was there evidence for haploinsufficiency that would have possibly indicated a contiguous gene syndrome. These findings are consistent with co-localization of distichiasis-lymphedema-cleft palate (DLC) with distichiasis-lymphedema syndrome in 16q24.3. Therefore, these two entities might be caused by mutations of a single, or of two closely-related gene(s).

Wolf-Hirschhorn/Pitt-Rogers-Danks phenotype with no detectable deletion. *F.F.B. Elder*¹, *G.N. Colasurdo*², *V.M. Rose*³. 1) Dept Pathology, The Univ TX Southwestern Med Ctr, Dallas, TX; 2) Div Pediatric Pulmonary Medicine, Dept Pediatrics, The Univ TX Houston Medical School, Houston, TX; 3) Div Medical Genetics, Dept Pediatrics, The Univ TX Houston Medical School, Houston, TX.

We present a patient with features consistent with Wolf-Hirschhorn/Pitt-Rogers-Danks (WHS/PRDS) phenotype but without a detectable deletion of 4p16.3 by either chromosome analysis or by fluorescence in situ hybridization (FISH). No deletions were found by FISH using a commercially available probe to the WHS critical region as well as 9 cosmid probes (provided by Dr. Michael R. Altherr, Genomics Group, Life Sciences Division, Los Alamos National Laboratory) spanning the WHS critical region. This 46 month old dysmorphic female from El Salvador presented with recurrent pulmonary infiltrates and failure to thrive. She was a term infant weighing 2.4 kg (5th%). She has short stature, microcephaly, moderate mental retardation with speech and language delay, a high forehead with a broad nasal bridge, telecanthus, slightly upslanting palpebral fissures, proptosis, simple protruding ears, short philtrum, wide mouth with down-turned corners and thin upper lip, micrognathia, and 5th finger clinodactyly bilaterally. She has not had seizures and does not have a cardiac defect.

Patients with PRDS have been demonstrated to have deletions of the 4p16.3 region, and PRDS is thought to be a less severe form of WHS. A few patients with PRDS but without detectable deletions have been reported. It is possible that these patients have a point mutation in the WHS gene. Our patient's phenotype appears to correspond with the milder end of the WHS/PRDS phenotypic spectrum.

Recurrent pulmonary infections and transient IgA deficiency have been described in a patient with WHS. Our patient had recurrent respiratory symptoms and interstitial infiltrates on chest radiographs. A lung biopsy revealed eosinophilic pneumonia which has not previously been associated with WHS. She was treated successfully with steroids.

Analysis of the p63 Gene in Classic EEC Syndrome, Related Syndromes, and Nonsyndromic Orofacial Clefts.

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EEC syndrome is an autosomal dominant disorder with the cardinal signs of ectrodactyly, ectodermal dysplasia, and orofacial clefts. Five families with EEC syndrome were recently linked to chromosome 3q27, and heterozygous p63 mutations were detected in nine unrelated EEC families (Celli et al, 1999). Homozygous p63 null mice exhibit craniofacial abnormalities, limb truncations, and absence of epidermal appendages such as hair follicles and tooth primordia (Mills et al, 1999; Yang et al, 1999). In this study, we screened 39 syndromic patients, including four with EEC syndrome, five with syndromes closely related to EEC syndrome, and 30 with other syndromic orofacial clefts and/or limb anomalies. We identified heterozygous p63 mutations in three unrelated cases of EEC syndrome, two Iowa Caucasian families and one sporadic case in a Filipino boy. One mutation ablates a splice acceptor site, and two mutations produce amino acid substitutions, R280C and R304Q, that alter conserved DNA binding sites. Germline mosaicism was detected in one case. The three cases demonstrate significant interfamilial and intrafamilial variability in expressivity. EEC patients with heterozygous p63 mutations presented with both isolated clefts of the secondary (soft) palate and clefts of the lip and primary (hard) palate. Genetics and embryology suggest that isolated clefts of the secondary palate may be a distinct clinical entity from clefts of the primary palate that involve the lip with or without the palate (Schutte and Murray, 1999), implicating p63 as an early gene in development prior to an important bifurcation in palatogenesis. We also screened p63 in 62 patients with nonsyndromic orofacial clefts, identifying an intronic single nucleotide polymorphism but finding no evidence of mutations that would explain even a subset of nonsyndromic orofacial clefts. This study supports a common role for p63 in classic EEC syndrome, both familial and sporadic, but not in other related or nonsyndromic forms of orofacial clefts.

Micrognathia, midfacial hypoplasia, hypotonia and severe developmental delay in brothers. A new X-linked or autosomal recessive condition? *R. Babul-Hirji*^{1,3}, *D. Chitayat*^{1,2,3}, *A. Toi*^{2,3}. 1) The Hospital for Sick Children, Toronto, ON., Canada; 2) The Mount Sinai Hospital, Toronto, ON., Canada; 3) The University of Toronto, Toronto, ON., Canada.

Although micrognathia is associated with numerous syndromes, the occurrence of severe micrognathia, developmental delay and hypotonia has not been previously reported. We report two brothers with the constellation of these findings. The first pregnancy was terminated after micrognathia was detected prenatally and the second pregnancy resulted in a newborn with micrognathia, hypotonia and severe delay. The mother was a 33-year-old primigravida woman and the father was 32 years old. The couple was non-consanguineous, healthy and their family histories were non-contributory. The first pregnancy was complicated with fetal ultrasound finding of severe micrognathia. The couple decided to terminate the pregnancy and this was done at 23 weeks gestation. Fetal autopsy revealed severe micrognathia, short forehead, midfacial hypoplasia and posteriorly rotated ears. The second pregnancy, which we did not follow, resulted in a newborn with severe micrognathia, posteriorly inserted tongue, high arched and intact palate and midfacial hypoplasia. He was hypotonic and later showed severe global delay. The brothers' karyotypes were normal and male, including FISH for 22q11.2 on the living male. Metabolic investigations done on the living brother showed no abnormalities. This is a hitherto new syndrome not reported previously that includes severe micrognathia with intact palate, midfacial hypoplasia and severe global developmental delay with hypotonia. The inheritance may be autosomal recessive or X-linked with prenatal diagnosis being possible.

Osteoglophonic dysplasia: Radiographic and pathologic features of a neonatal lethal form. *D.K. Grange, D.S. Brink, A. Luisiri, F. Sadiq, C. Sotelo-Avila.* Saint Louis University School of Medicine, St. Louis, MO.

We report an infant with a lethal form of osteoglophonic dysplasia, an autosomal dominant skeletal dysplasia characterized by craniosynostosis, rhizomelic shortening of limbs, lucent metaphyseal defects, bowing of long bones, and vertebral abnormalities. Lytic bone lesions contain fibrous tissue. The patient was born at 36 weeks gestation to normal parents. She had macrocephaly and a Kleeblattschaedel skull deformity, a tiny thorax and marked rhizomesomelic shortening of the limbs. Facial features were coarse with proptosis, depressed nasal bridge, protruding tongue and severe micrognathia. Respiratory failure lead to death at age 4 days. Radiographs showed craniosynostosis of coronal, sagittal, parieto-mastoid and squamosal sutures. There was extreme hypoplasia of the mandible. The ribs were very short and clavicles were markedly hypoplastic. Bowing of the femurs and tibiae was present, with an apparent fracture of the right femur. There was endosteal thickening of the medial cortex of the long bones. Proximal metaphyses of both humeri had cleft-like lesions. There was brachydactyly with angel-shaped phalanges, seen in only one of the eight previously reported cases of osteoglophonic dysplasia. Lytic lesions of the proximal and distal metaphyses were present in phalanges, metacarpals and metatarsals. On pathological examination, there was abnormal endochondral ossification with poorly formed physes. Fibrous tissue was present within the metaphyses and diaphyses of multiple bones. In the phalanges, the bone trabeculae were dense and the metaphyseal lytic lesions corresponded to replacement of bone by masses of fibrous connective tissue. In the right femur, in the region of the apparent fracture, continuity of bone was interrupted by fibrous connective tissue and osteoid in a fibrous dysplasia-like pattern. The distal epiphysis and metaphysis of the humerus had a large synovial-lined cyst that communicated with the joint space and was surrounded by dense fibrous tissue. This case expands the spectrum of the radiographic and pathologic features of osteoglophonic dysplasia and may contribute to the understanding of the pathogenesis of this rare disorder.

Ellis van Creveld syndrome (chondroectodermal dysplasia MIM 22550) in three siblings from a non-consanguineous mating. *E. George*¹, *E. Lieber*^{1,2}. 1) Department of Pediatrics, New York Methodist Hospital, Brooklyn, NY; 2) Department of Medicine(Division of Genetics), New York Methodist Hospital, Brooklyn, NY.

Ellis van Creveld syndrome (EvC), is a rare autosomal recessive condition. Approximately 200 cases have been described in the literature - half of them within a subgroup of the Amish population in Pennsylvania. The syndrome includes features of acromelic and mesomelic shortening of long bones, postaxial polydactyly, nail dysplasia, and dental anomalies. We describe three siblings with EvC from a non-consanguineous mating of Ashkenazi Jewish origin. All the siblings had classic features of EvC. Two of the patients had complications that are seen in EvC. The first daughter died at nineteen days of life with respiratory failure secondary to a narrow rib cage. The second daughter underwent open-heart surgery for repair of atrial septal defect. The third daughter, the proband, was diagnosed prenatally with fetal ultrasonography at 25 weeks of gestation. The sonogram revealed short extremities, a narrow rib cage, and polydactyly. The infant was born by vaginal delivery at 42 weeks of gestation. She is scheduled for surgical removal of bilateral extra digits and extraction of natal teeth. The differential diagnosis of EvC includes Jeune's asphyxiating thoracic dysplasia (JATD), Weyers acrodistal dysostosis, and short rib polydactyly syndrome. The mutant gene in individuals with EvC has recently been identified on chromosome 4p16, proximal to other genes associated with conditions of short stature (classic achondroplasia, Weyers acrodistal dysostosis) and other skeletal disorders. Five different mutations have so far been characterized. It is plausible to assume, that a range of mutations in the critical gene is responsible for the variation of phenotypic expression in EvC.

A new association comprising distinctive facies, chest deformity and camptodactyly with normal thrive and normal intelligence. *H. Chaabouni*^{1,2,3}, *M. Chaabouni*^{2,3}, *F. Maazoul*³, *N. Smaoui*^{2,3}, *L. Ben jema*^{2,3}, *R. Mrad*^{2,3}. 1) El Menzah 1XA, Tunis, Tunisia; 2) Laboratory of Human Genetics. Medical School Tunis, Tunisia; 3) Department of Hereditary Diseases .Charles Nicolle hospital. Tunis,Tunisia.

We report the clinical findings in a 14 years-old Tunisian boy with distinctive facial dysmorphism, chest deformity ; camptodactyly , with normal stature and normal intelligence. Abnormal facies findings are proptosis, down-slanting palpebral fissures, hyperterolism, broad nasal tip with large nostrils, hypoplastic maxillary with thin upper lip, delayed eruption of teeth , dental malocclusion , high narrow palate and malformed ears. The skull is characterised by flat occiput. The child has severe pectus excavatum and bilateral camptodactyly of second, third and fourth fingers.External genitalia examination is normal. Radiographic studies of hands, feet, skull and chest showed reported deformities without bone or cartilage lesion.The karyotype is normal. The clinical features have been observed in neonate and there is no evolutive variation. His mother has few same features : proptosis ,dental malocclusion, thin upper lip and she has partial conductive deafness. This pattern is concordant with dominant inheritance and variable expression of the syndrome. We suggest that this is a new association that has not been described previously in the literature.

Symptoms of Schizophrenia in adults with 22q Deletion Syndrome. *A.S. Bassett¹, E. Chow¹, L. Scutt¹, P. AbdelMalik¹, K. Hodgkinson¹, R. Weksberg².* 1) Dept Psychiatry, CAMH, QSD, Univ Toronto, Toronto, ON, Canada; 2) Division of Clinical and Metabolic Genetics, HSC, Toronto, ON, Canada.

22q Deletion Syndrome (22qDS), including velocardiofacial and DiGeorge syndromes, is associated with 22q11 deletions, and has an increased prevalence of schizophrenia (SZ). Objective: To determine whether the pattern of schizophrenic symptoms differed in 22qDS from other forms of SZ and whether childhood functioning was associated with outcome in adults with 22qDS. Methods: 17 subjects with 22qDS and SZ were compared with 16 age- and sex-matched subjects from a familial SZ sample. All subjects were assessed for symptoms of schizophrenia using a standard scale (PANSS); 22qDS-SZ subjects were also assessed using the Premorbid Adjustment Scale for social functioning in childhood/adolescence. Results: There were no significant differences between 22qDS-SZ and familial SZ groups in median age at onset of schizophrenia (20 y vs. 22 y), global functioning, or negative (e.g., social withdrawal) or mood (anxiety/depression) symptom scores. The 22qDS-SZ group had higher excitement symptom (e.g., poor impulse control) ($z=2.5$, $p=0.01$) and cognitive (e.g., abstract thinking) ($z=3.2$, $p=0.001$) symptom scores. In the 22qDS-SZ group there was a significant correlation between poor childhood social functioning and negative symptoms in adulthood ($r=0.67$, $p=0.008$). Conclusions: The results suggest that 22qDS-SZ may not be easily distinguishable in SZ populations based on clinical psychiatric symptoms. Poor childhood social functioning may be associated with enduring symptoms in individuals with 22qDS who develop schizophrenia.

Hypoparathyroidism: The link between femoral-facial syndrome and maternal diabetes. *B.N. Chodirker^{1,2}, A. Chiu¹, S.M. Menticoglou³*. 1) Pediatrics and Child Health, University of Manitoba, Winnipeg, MB., Canada; 2) Biochemistry & Medical Genetics, University of Manitoba, Winnipeg, MB., Canada; 3) Obstetrics and Gynecology, University of Manitoba, Winnipeg, MB., Canada.

A 36 year old Aboriginal Canadian woman was seen at 29 weeks gestation when a prenatal ultrasound examination documented significant fetal femoral bowing. The femurs appeared thin and shortened. No other fetal anomalies were identified at that time. Family history was unremarkable. She was on insulin as she had been diabetic since age 19. Her diabetes control was poor. The initial prenatal blood glucose at 10 weeks gestation was 17 mmol/L. Biochemical testing showed that she had normal serum levels of calcium, phosphate, alkaline phosphatase and 25-hydroxy vitamin D. Her parathyroid hormone (PTH) level was low at 5 ng/L (normal range 12-72). At 36 weeks, she delivered an infant girl with typical features of femoral facial syndrome (FFS). Birth weight was 3480 grams (95 %tile). There was significant micrognathia. There was a cleft of the soft palate. The baby required intubation after birth due to airway obstruction. Skeletal survey confirmed short bowed femurs but did not detect any other significant anomaly. Neonatal problems included hypoglycemia, hypocalcemia and hypophosphatemia. Serum magnesium was low normal at 0.62 mmol/L (normal 0.5- 1.2). A serum PTH level was initially low normal (15 ng/L). The medical literature supports the following: Infants of diabetic mothers (IDM) are at increased risk for various congenital anomalies e.g. caudal regression. These are thought to result from poor glucose control early in pregnancy. FFS is associated with maternal diabetes. PTH levels are lower in diabetic women in pregnancy compared to non-diabetic women. Hypocalcemia in IDM is thought to be due to failure to increase PTH synthesis after birth possibly secondary to hypomagnesemia. Bone mineral density is significantly decreased in infants of diabetic mothers. Fetal parathyroids are needed to maintain increased calcium levels and stimulate fetal osteoblasts and bone mineralization. These findings along with the features of this case support a role for hypoparathyroidism in the pathogenesis of FFS.

The adult phenotype of Meier-Gorlin syndrome (ear-patella-short stature syndrome) with comparisons to previous cases. *G.C. Gowans¹, M. Pelletier¹, M. Tanzer², F.C. Fraser¹.* 1) The F. Clarke Fraser Clinical Genetics Unit, Departments of Pediatrics and Human Genetics, Montreal Children's Hospital and McGill University, Montreal, QC, Canada; 2) Department of Orthopaedics, Montreal General Hospital, McGill University, Montreal, QC, Canada.

We report a 39-year-old man of normal intellect with microcephaly, a small triangular-shaped face, bilateral microtia, maxillary and mandibular hypoplasia, unilateral cryptorchidism, and absent patellae among other anomalies. The proportionate short stature, characteristic and distinct craniofacial appearance, small, simple and low-set ears, and absence of patellae correspond to most previous reports of Meier-Gorlin syndrome (MGS) or Ear, Patella, Short stature syndrome (MIM #224690). However, with only approximately a dozen cases of this syndrome in the literature, there has been some question as to what constitute "cardinal" signs or symptoms. Some cases included as representative of MGS do not have the distinctive small and simple ears reported in the original patient of Meier et al. (1959) or in the second patient reported by Gorlin (1975 and 1992). The absence of patellae reported in these original two patients are not obligate findings since hypoplastic and normal patellae have been found in patients considered to have MGS. In addition, the adult phenotype of MGS has rarely been described and therefore the natural history of clinical manifestations is not well known. We compare the clinical manifestations of our patient with those reported in children and adults thought to have this short stature syndrome in order to further delineate MGS. We also describe the knee arthroplasty used to treat our patient.

Pleiotrophy or coincidence: unusual findings in DiGeorge/velocardiofacial syndrome (DGS/VCFS). *L.P. Cook¹, L.E. Walsh^{1,2}, J.K. Hartsfield, Jr.^{1,3}*. 1) Dept Med & Molec Genetics, Indiana Univ Sch Med, Indpls., IN; 2) Dept Neurology, Indiana Univ Sch Med, Indpls., IN; 3) Dept Oral-Facial Development, Indiana Univ Sch Dentistry, Indpls., IN.

We report two patients with DGS/VCFS who also had findings not usually associated with this disorder. Patient 1 was a 2-week-old boy born after pregnancy complicated by gestational diabetes requiring insulin. Physical findings included downslanting palpebral fissures, prominent nose, micrognathia with intact palate, dysplastic ears, systolic murmur, closed omphalocele with large inguinal hernias, camptodactyly, and bilateral equinovarus deformity. Initial karyotype (46, XY), head CT scan, and renal ultrasound were normal. Echocardiogram showed an abnormal right subclavian artery. Subsequent evaluation of the child's mother revealed history of depression, hypernasal speech, ventriculoseptal defect (VSD), and borderline hypocalcemia. DGS FISH analyses demonstrated 22q11 deletion in both child and mother. Patient 2 was a 6-year-old boy, the product of an unremarkable pregnancy. By age 6 months, he was found to have ASD and VSD. Recurrent infections and hypocalcemia prompted discovery of a hypoplastic thymus and parathyroid glands. His facial features suggested DiGeorge syndrome, but his mother deferred further genetic testing. At age 3 years, he was developmentally delayed, especially in language, borderline microcephalic, and preferentially used his left hand. A brain MRI showed an isolated Dandy-Walker malformation. At age 5 years, he was microcephalic, spoke 6 words, but used sign language well. DGS FISH analysis revealed microdeletion of 22q11. Karyotype was normal (46, XY) otherwise. Omphalocele has not been reported previously in the literature with VCFS; Dandy-Walker malformation has been reported once in DiGeorge syndrome complicated by further findings of CHARGE association. It is possible that our patients' unusual manifestations were caused by their underlying microdeletions. The presence of such dramatic malformations may overshadow more subtle, but more diagnostic abnormalities in DGS/VCFS and should not preclude specific genetic testing for the disorder.

Are Hypotrichosis Simplex and Monilethrix Allelic Forms?: Description of a Mexican family. *N. Davalos^{1,2,3}, I.P. Davalos², B. Patiño-Garcia^{1,2}, A. Garcia-Vargas³, V. Tarango-Martinez³, M.E. Sanchez-Castellanos³, I. Villarreal³, D. Garcia-Cruz^{1,2}, A. Feria-Velazco⁴, J.M. Cantu^{1,2}, L.E. Figuera^{1,2}.* 1) Div. Genetica y Medicina Molecular, CIBO-IMSS, Guadalajara, Mexico; 2) Doctorado Genetica Humana, CUCS, UdeG, Guadalajara, Mexico; 3) Instituto Dermatologico de Jalisco Jose Barba Rubio. Guadalajara, Jalisco Mexico; 4) CIATEJ, Guadalajara, Jalisco, Mexico.

We describe a Mexican family with 19 affected individuals through five generations, presenting a hair characterized by defect limited to the scalp. Clinically the 11 year-old proposita was normal at birth. Between 9-10 years of age displayed hypotrichosis. The hairs were sparse, thin, light, and short and were not easily plucked. Dental defects, other nail or sweat gland disorders and mental retardation were excluded. Psychomotor development was normal. Laboratory tests including complete blood count, urine analysis, metabolic screening were all in the normal range. Karyotype analysis gave no evidence of chromosomal abnormalities. Microscopic examination of the hair showed delicate hairs with irregular pigment distribution, structural defects varying reduced shaft diameters. The other 18 affected individuals (ranging from 11 to 60 years of age) were examined, showing similar characteristics, and no other associated features, all of them had onset of the trait at 8-11 years of age. Hypotrichosis is a common feature of many inheritable disorders, among them are hypotrichosis simplex and monilethrix. They have been differentiated based on the beading of the hair only present in monilethrix. The family here presented has shown an irregular diameter pattern in some of the analyzed hair shafts, but not in others. Given the intermediate clinical features displayed by the affected individuals we propose both entities could be considered alleles having different mutations at the same locus.

Assessment of variation in clinical outcome and mechanisms underlying mosaic Down Syndrome. *N. Gursoy¹, G. Hill¹, A. Gregory^{1,2}, B. Landa¹, A. Pandya¹, L. Vanner¹, C. Jackson-Cook¹.* 1) Department of Human Genetics, Medical College of Virginia Campus, Richmond, VA; 2) DNA Dynamics, Inc., Del Mar, CA.

Mosaicism for trisomy 21 is seen in only 1%-2% of all individuals with Down syndrome. Factors contributing to variations in phenotype for people with mosaic Down syndrome (MDS) are not well understood. To assess this variability in clinical outcome and mechanism underlying MDS, we performed a multidimensional project. To date, we have studied 44 cases of MDS (along with their parents and sibs), ranging in age from 4 months to 33 years. The information collected for the MDS cases included the: (1) proportion of lymphocytes (cultured and uncultured) and buccal mucosa cells that were trisomic; (2) physical and developmental outcome; and (3) cellular timing of the malsegregation resulting in the observed mosaicism. Using FISH to enumerate the chromosomes 21, no significant difference in the percentage of cells having a trisomic complement was detected between cultured and uncultured lymphocytes. In contrast, the proportion of trisomic cells in the buccal mucosa cells was different from that of lymphocytes ($p < 0.001$), with a trend toward higher levels of trisomic cells being seen in the buccal cells. The proportion of trisomic cells in our cohort ranged from 16%-97%. In general, the highest level of trisomy identified (either in lymphocytes or buccal smears) led to a higher correlation with clinical outcome. A trend toward increased severity was noted in the individuals having higher levels of trisomic cells. In particular, two-thirds (7/11) of all patients having congenital heart anomalies had trisomy levels of $>75\%$. Differences in the chromosomal malsegregation events leading to mosaicism, assessed using SSR DNA markers, were also seen and included cases arising from (1) both a meiotic and mitotic nondisjunctional event; or (2) a single mitotic error. To date, no clear correlation has been detected between clinical outcome and mechanisms of the chromosomal malsegregation. In summary, by studying a large group of individuals with mosaicism we hope to improve our understanding of the observed diversity in people having MDS, as well as the causes of chromosomal malsegregation.

A new MR syndrome associated with bilateral global colobomas, cleft palate, cerebral, skeletal and genital anomalies in three brothers. *S. Cappon, M. Khalifa.* Dept Pediatrics and Pathology, Queen's University and Kingston General Hospital, Kingston, Ontario, Canada.

We describe three Native Canadian brothers of a sibship of six with a previously undescribed pattern of malformations, including bilateral colobomas, cleft palate, craniofacial anomalies, skeletal defects, hypospadias with bifid scrotum and severe psychomotor retardation. The proband is currently five years old and has an associated heart defect (VSD and mild right pulmonary venous stenosis). Eye examination revealed bilateral iris and chorioretinal colobomas and right optic disc coloboma. MRI of the head was significant for polygynea, cortical dysplasia and shallow orbits. Hearing test was normal although no speech has developed to date. The chromosomes were normal. The next son was full term and had identical facial, cranial, skeletal and genital anomalies. He also had a diaphragmatic hernia, identified prenatally. His chromosome study was normal as well as FISH for 22 deletion. He died shortly after birth because of pulmonary hypoplasia. The third son is ten months old with the same facial anomalies, colobomas, cleft palate, triangular face, widely separated sutures, skeletal and genital anomalies. A skeletal survey showed brachycephaly, metaphyseal flaring, wormian bones, and 11 sets of ribs. His cytogenetic study was normal. To our knowledge, this constellation of abnormalities has not been described before. The inheritance pattern could be consistent with an X-linked or autosomal recessive disorder.

von Voss-Cherstvoy syndrome: A second family with affected siblings. *N.S.N. Gilpin.* Dept Pediatrics, Christie Clinic Assoc, Champaign, IL.

von Voss-Cherstvoy syndrome, sometimes referred to as DK Phocomelia, is a multiple congenital anomaly syndrome of occipital encephalocele, radial ray defects and urogenital anomalies. This report describes an Amish family with 2 consecutive affected children. This is the second family reported with affected siblings, supporting the theory of autosomal recessive inheritance and allowing further delineation of the syndrome. Development appeared arrested at 18 to 20 weeks gestation. Chromosome and platelet studies were normal. von Voss-Cherstvoy syndrome is attributed to a gene controlling early development.

The association between paternal alcohol exposure and Turner syndrome(TS): Preliminary results of a health and lifestyle survey of parents of children with TS. *S. Kagan¹, S. Vohra¹, G. Koren¹, P. Selby^{1,2}.* 1) Department of Clinical Pharmacology and Toxicology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Center for Addiction and Mental Health, Toronto, Ontario.

TS is a sex chromosome aneuploidy that occurs as a result of a nondisjunctional error in meiosis I or anaphase lag; however, the etiology of this disorder remains unknown. Anecdotal evidence suggests that paternal alcoholism may play a role in the etiology of Turner syndrome (TS). Accordingly, the objectives of this study were: (1) to determine the potential association between paternal alcohol exposure and TS; and (2) to determine the potential association between selected health and lifestyle behaviors of the parents and TS. This descriptive study employed a self-report survey methodology. The questionnaire was designed to solicit information about the parents' health and lifestyle habits occurring one year prior to and throughout the pregnancy of their daughter with TS. The primary outcome measure included in the questionnaire was the Brief Michigan Alcohol Screening Test (BMAST). The remainder of the questionnaire included questions about 5 other health and lifestyle behaviors that were determined by clinical experts to be relevant to the objectives of this study. The study population was solicited from the Turner's Syndrome Society of Canada and included any parent(s) having a child with TS who was of any age. The questionnaires were mailed to 245 families and 212 families completed and returned the survey (86.5% response rate). Six of the fathers (3.6% n=166) and six of the mothers (3.6% n=165) had scores of 5 or more on the BMAST (scores of 5+ are considered to be in the "alcoholic range"). This is considerably lower than the population norm of 9.5%. An interesting finding suggested that 54% of the fathers and 36% of the mothers reported that they had been exposed to two or more environmental hazards. Our study has not indicated that there is a potential association between paternal or maternal alcohol consumption and TS. However, we were surprised to learn that a previously unidentified association between exposure to environmental hazards and TS may exist.

Unilateral ocular malformations, postaxial polydactyly and delayed intramembranous ossification; a new autosomal dominant condition. *D.M. Martin¹, J.L. Gorski^{1,2}*. 1) Department of Pediatrics, The University of Michigan, Ann Arbor, MI; 2) Department of Human Genetics, The University of Michigan, Ann Arbor, MI.

Molecular genetic analysis has substantially enhanced our understanding of eye embryogenesis. Uniformly, of the genes identified in ocular development, mutations result in bilateral malformations and disease. Here we present a family segregating unilateral ocular malformations in association with postaxial polydactyly and delayed intramembranous ossification. This family was referred for evaluation of multiple congenital anomalies. The father exhibited unilateral microphthalmia, retinal coloboma, scleralized cornea and abnormal iris, nonosseous unilateral postaxial polydactyly, and delayed closure of the anterior fontanelle. His growth and cognition were normal. His first son, age 2 at initial presentation, displayed unilateral postaxial polydactyly, delayed closure of the anterior fontanelle, and unilateral talipes equinovarus requiring surgical correction, with normal growth and development. His second son, age 5 months, presented with unilateral congenital glaucoma, postaxial polydactyly, and a large anterior fontanelle. Skeletal survey revealed delayed intramembranous ossification. No shortened or abnormally formed skeletal elements were identified. His growth and development were normal. Both sons had normal peripheral karyotypes and thyroid function tests. We conclude that this family appears to be segregating a trait that adversely affects ocular development and intramembranous ossification, with male to male transmission suggesting autosomal dominant inheritance.

New Family Member in Previously Reported Autosomal Dominant Vertebral Hypersegmentation Pedigree Increases Likelihood of Association with Cleft Lip and/or Palate. *K.B. Nash, A. Sommer, A.C. Lidral.* Ohio State University and Children's Hospital, Columbus, OH.

Autosomal dominant vertebral hypersegmentation has only been described in 1 family (Sommer, 1993). The male proband with 8 cervical and 6 lumbar vertebrae and 14 ribs was ascertained at birth upon chest X-ray for hyaline membrane disease. The family reported that the proband's father and the father's sister also had extra ribs. Subsequent X-ray of the father showed identical hypersegmentation of vertebrae and ribs as the proband. The aunt reports that her extra ribs were confirmed at age 9 y. when she was X-rayed after an accident. Her son also appears to be affected with a disproportionately long torso. An interview with the paternal grandmother revealed that she too had the same hypersegmentation. At that time, a complete work up of the proband revealed no additional abnormalities. The affected relatives reported no other clinical manifestations, except that the father had unilateral left cleft lip and palate (LCL+P). Since cleft lip with or without cleft palate (CL±P) is a common birth defect (1/1000), it was unclear whether this was an incidental finding representing nonsyndromic CL±P.

We now report on the proband's new sister who has the same radiological findings of 14 thoracic vertebrae and ribs, as well as LCL+P. In addition, the affected aunt reports having had a pharyngeal flap put in place at age 21 y. to improve hypernasal speech, which may be a manifestation of the cleft palate spectrum (CP). Nonsyndromic CL±P and CP are distinct genetic entities, occurring at different times during development, and usually not occurring in the same family. To the contrary, several pleiotropic syndromes show CP or CL±P occurring within families.

In summary, we report a family with apparent autosomal dominant hypersegmentation with 2 of the 5 affected members having overt cleft lip and palate and another with symptoms often associated with cleft palate. It is unclear whether these are two independent traits segregating in the family or whether the two traits are etiologically related.

Partial trisomy 12 and 13 in a newborn. *J.G Habecker-Green¹, V.L Ramie¹, R. Naeem³, L. Bayer-Zwirello², G.M Cohn¹.* 1) Clinical Genetics, Baystate Medical Center, Springfield, MA; 2) Maternal Fetal Medicine, Baystate Medical Center, Springfield, MA; 3) Cytogenetics, Baystate Medical Center, Springfield, MA.

We present a case report of a unique chromosomal syndrome. A 25-year-old, pregnant female of Puerto Rican ethnicity presented to genetics at 28 weeks gestation for the ultrasound finding of fetal tetralogy of Fallot and a vertebral anomaly. History information indicated that she had had three unexplained first trimester pregnancy losses, and had never been evaluated for peripheral karyotype. Amniocentesis was discussed and declined. Karyotypic analysis of the patient was discussed and accepted. The patient's karyotype was 46,XX,t(12;13)(q24.3;q14). This information was discussed with her, along with the possibility that the fetal karyotype could be unbalanced. The patient chose to analyze the fetal karyotype through amniocentesis. Fetal karyotype was 47,XX,+der(13)(12;13)(q24.3;q14). It was discussed with the patient that previous case reports of this chromosomal anomaly could not be located, so prognostic information was limited. However, with large, unbalanced chromosomal segments, we expected a greatly increased risk for neonatal mortality and for mental retardation in survivors. This and her options for neonatal management were discussed with the mother. The mother chose to have her child treated as clinically indicated.

The infant was born at 39.6 weeks gestation, and required resuscitation. She weighed 5 lb 5 oz, and the following features were noted: posteriorly sloping forehead with midline crease, broad nasal root, micrognathia, excess nuchal skin, generalized loose skin, fistled hands, diastasis recti, a prominent clitoris, and a right rocker bottomed foot. Renal, head, and abdominal ultrasounds were normal. Echocardiography confirmed tetralogy of Fallot. Neurology evaluation was significant for hypotonia. Currently the child is 6 months of age and has developmental delays and growth retardation. Other case reports of either patient's karyotype have not been located.

A new genetic malformation syndrome characterized by microcephaly, jejunal atresia, and XY sex reversal. *C.E. Keegan, J.W. Innis.* Dept Ped Gen, 1924 Taubman Ctr, Univ Michigan, Ann Arbor, MI.

The patient is a female infant born at 32 weeks gestation to a non-consanguineous couple by spontaneous vaginal delivery. She was evaluated by Genetics secondary to microcephaly, with a birth OFC of 25 cm, and jejunal atresia. No other dysmorphic features were noted on physical examination. Karyotype revealed a 46 X,Y male chromosomal complement. Endocrinologic evaluation revealed testosterone levels in the normal range for a male infant. Androstenedione, cortisol, DHEA, 17-OH pregnenolone, and 17-OH progesterone were normal. Ultrasonography and MRI revealed a primitive gonad on the left and a retrovesicular fluid-filled cyst, most likely a Mullerian remnant. No definitive uterus or ovaries were identified. Jejunal atresia was repaired in the neonatal period by excision of the atretic segment and end-to-end anastomosis. Upon re-evaluation at age 7 months, her head circumference was still less than the 5th centile at 36 cm. Genitourinary exam showed clitoromegaly. Development was normal for corrected gestational age. A high-resolution prometaphase karyotype was normal. SRY mutational testing and 7-dehydrocholesterol are currently pending. Removal of the primitive gonad is planned. The sex reversal phenotype in this patient is most consistent with mixed gonadal dysgenesis although partial androgen insensitivity cannot be excluded. This constellation of findings represents a previously undescribed syndrome associated with sex reversal.

Facial Dysgenesis: A new facial syndrome with absent eyes and palpebral fissures, presence of proboscis, square stoma and clefting. *J.E. Hoover¹, J. Cai¹, C.B. Cargile², G.H. Thomas², E.W. Jabs¹, A. Hamosh¹*. 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Kennedy Krieger Institute, Baltimore, MD.

We report a female infant with primary anophthalmia and cryptophthalmos, temporal remnant "eye tags", bilateral cleft lip, unilateral cleft palate, choanal atresia, bilateral external auditory canal atresia, grossly normal brain by MRI and an interstitial deletion of chromosome 7. The pregnancy was complicated by polyhydramnios, breech presentation, and premature rupture of membranes and fetal bradycardia at 33 weeks gestation necessitating C-section. Her birth weight and head circumference were at the 5%tile. She transferred to our institution at 22 days of life with a tracheostomy and poor gain in weight, length and head circumference. Grossly her limbs, trunk and genitalia were normal. Skeletal survey, echocardiogram and abdominal viscera were normal except for a split central sinus of the right kidney. BAER exam indicated she could hear and temporal CT confirmed the presence of cochlea and possible ossicles. She died at six months of age from sepsis/airway obstruction precluding evaluation for craniosynostosis or cognitive impairment. Cytogenetic studies revealed a deletion of chromosome 7, del(7)(p15.1p21.1) at 650-band resolution. By FISH using BAC370M10 (188 kb) as a probe, one allele of the TWIST gene was found to be deleted. No mutations were detected upon sequencing the coding region of the other TWIST allele. Parental karyotypes and phenotypes were normal. There are no other reported patients with bilateral anophthalmia, cryptophthalmos and a grossly normal brain, limbs and genitalia. Furthermore, patients with chromosome 7 deletions that overlap or include that of our patient do not have anophthalmia, cryptophthalmos or external auditory canal atresia. These data suggest that this clinical presentation, which is similar to but distinguishable from Fraser syndrome, may be caused by a bi-allelic mutation in this interval in a single gene other than TWIST.

Phenotypic characteristics of Warrdenburg syndrome type I and type III in a family. *D. Lin, S. Lin.* Dept Pediatrics, Mackay Memorial Hosp, Taipei, Taiwan.

Warrdenburg syndrome is an autosomal dominantly inherited disorder with variable penetrance. The major characteristics features are as follows: 1)Dystopia canthorum; 2)synophrys; 3)broad nasal root 4)pigmentation of hair , skin, or both; 5)heterochromic or hypochromic irides; 6)congenital deafness. Genetic heterogeneity has led to classification of affected families as type I (WS-I), with dystopia canthorum, or type II, without dystopia canthorum. Klein-Warrdenburg syndrome (WS-III) is a disorder with many of the same characteristics as WS-I and includes musculoskeletal abnormalities. We report otherwise typical WS-I and WS-III in a family, 3 females with WS-I and 2 males with WS-III. All affected members demonstrated dystopia canthorum, synophrys, and broad nasal root, and both WS-II had clinodactyly and contracture digits. Other characteristics features including: hypoplastic blue irises affected 1 WS-I and both WS-III, a unilateral sensorineural hearing loss in both WS-III and a bilateral sensorineural hearing loss in 1 WS-I, and pigmentary disturbance in 1 WS-III. To our knowledge, both WS-I and WS-III in a family has not been previously reported in Warrdenburg syndrome.

Molecular Studies in Patients with Rare Craniosynostosis Syndromes: Pseudoaminopterin, Shprintzen-Goldberg.

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44% of patients with craniosynostosis seen in the Craniofacial Clinic at CHOP were noted to have classical syndromes and have a confirmed molecular etiology. Amongst the remaining patients, after particular attention to limb abnormalities, two rare clinical syndrome diagnoses have emerged. Patient 1, a full term product of an uncomplicated pregnancy had turriccephaly with bicoronal synostosis and partial closure of the right lambdoidal suture. Partial soft tissue syndactyly of fingers 2, 3, 4 and a proximally placed thumb were seen bilaterally. There was only a single digit with a single metatarsal bilaterally. The findings were similar to cases of exposure to aminopterin and cases termed aminopterin like syndrome without aminopterin; the latter most likely here. Patient 2 was noted to have hypotonia at birth and turriccephaly, wide set eyes, exorbitism, hypoplasia of the maxilla and mandible, one large fontanel in midparietal region, coronal synostosis, partial lambdoidal stenosis and long fingers and toes. On a follow-up exam at 19 months, the loose skin, high myopia, umbilical and inguinal hernia, together with the craniosynostosis suggested the diagnosis of Shprintzen-Goldberg syndrome. Lower extremity bowing and metatarsus adductus, bowing of the ulna, a short radius, dislocation of the radial heads, 13 ribs, and square box like vertebrae were noted, all consistent with this diagnosis. A cardiac evaluation revealed mild dilatation of the aortic root. Since craniosynostosis is a major component of each syndrome and the majority of cases were reported before the advent of molecular diagnostics for craniosynostosis syndromes, we performed molecular analysis including FGFR1, 2, 3, and TWIST in both of our patients, and deletion studies for the TWIST gene in patient 2. All of the studies were normal. Thus, the etiology of these syndromes remains unknown.

Partial trisomy 2q:two new cases. *M.E. Mollica¹, N. Sganzzetta¹, M. Aguirre¹, L. Lopez Miranda¹, S. Acevedo², P. Barbero¹.* 1) Centro Nacional de Genetica Medica Buenos Aires, Argentina; 2) Academia Nacional de Medicina.Buenos Aires, Argentina.

Partial trisomy of the distal part of the long arm of chromosome 2 is a rare condition with craniofacial anomalies and visceral abnormalities associated with psychomotor retardation and developmental delay. We report on two female infants with multiple dysmorphic features and partial trisomy of 2q. Patient 1:age 11 months. Clinical findings: brachycephaly, prominent forehead, hypertelorism, depressed nasal bridge with anteverted nares, long philtrum, low set ears, tent-shaped mouth, micrognathia, short neck, single bilateral palmar creases, brachydactyly, clinodactyly of the fifth fingers, cardiac and genital abnormalities and intestinal atresia. Her karyotype was 46,XX,der(15)t(2;15)(q35;p12)mat. Patient 2:age 3months. Clinical findings: brachycephaly, prominent forehead, hypertelorism, depressed nasal bridge with anteverted nares, long philtrum, low set ears, big mouth, short neck, brachydactyly, clinodactyly of the fifth fingers, congenital cardiac abnormalities and polycystic kidney. Her karyotype was 46,XX,der(9)t(2;9)(q33;p12)mat. In both of them partial trisomy 2q was confirmed by FISH using whole painting chromosome 2 (WCP 2). Patient 1 presents a pure 2q trisomy, in patient 2 despite of the associated 9p monosomy the clinical features are more concordant with those of trisomy 2q. Although the severity of the clinical manifestations in previous reports appears to be related with trisomy of the proximal region of 2q33, the internal malformation in our cases seem to be related with the duplication of distal loci.

Clinical manifestations of Marfan syndrome in Hispanic families. *F.A Laws¹, D. Guo¹, S. Kuang¹, C.A. Morris², D.M. Milewicz¹.* 1) Internal Medicine, UT-Houston Medical School, Houston, TX; 2) Clinical Genetics, Univ NV Sch Med, Las Vegas, NV.

Marfan syndrome (MFS) is an autosomal dominant condition with pleiotropic manifestations involving the skeletal, ocular, and cardiovascular systems. The diagnosis is primarily a clinical diagnosis based on involvement of these and other systems, referred to as the "Ghent criteria". We have identified 2 Hispanic families with aortic root dilatation and dissection inherited in an autosomal dominant manner. The first family had 12 affected and 10 unaffected members available for assessment. FBN1 sequencing of DNA from affected individuals revealed a frameshift mutation in exon 24 (3074delT). Fibroblasts explanted from an affected member were metabolically labeled to study fibrillin-1 (fib-1). These studies revealed diminished amounts of fib-1 synthesized and <5% fib-1 deposited in the extracellular matrix when compared with control cells. Assessment of the clinical features in 8 affected adults in the 3rd and 4th generation revealed that none of the affected individuals had major skeletal manifestation of MFS. Only 3 of the 8 meet the criteria for involvement of the skeletal system. None of the individuals have involvement of the ocular system. A second Hispanic family with aortic root dilatation and dissection had 7 affected and 9 unaffected members. The cardiovascular phenotype was linked to markers within and near FBN1. Two affected adults were examined and only one had skeletal system involvement. Two adults with the affected haplotype were assessed by local cardiologists and reported to have no skeletal features of the MFS. The family has not been examined for ocular manifestations. Other large Hispanic MFS families have not been identified. The results with these 2 families suggest that Hispanic individuals may not manifest skeletal features of the MFS to the same extent as Caucasians, indicating that the Ghent criteria for diagnosing MFS may need to be revised for this ethnic group.

A new Weaver-like syndrome with osteochondroma and epiblepharon. *D. Wattanasirichaigoon¹, N.*

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Weaver syndrome is a rare condition of macrosomia, accelerated skeletal maturation, camptodactyly, flared metaphysis, characteristic facial appearance, and mental retardation. We describe an 11 year-old girl who had features of Weaver syndromes, including facial phenotypes, postnatal somatic overgrowth, omphalocele, contracture of interphalangeal joints and thin deep-set nail, but metaphyseal splaying. In addition, the patient possessed congenital epiblepharon and osteochondroma at her both proximal ends of tibias. These clinical findings have not been reported in Weaver syndrome. After comparing Weaver syndrome with and contrasting it to other disorders, we concluded that novel findings in this patient could represent a new Weaver-like disorder.

A de novo mutation [45,XX,der(5), t(5;14) (pter;q11.2)] and a neuroectodermal disorder, mimicking the MLS phenotype. *R. Zannolli¹, R. Mostardini¹, L. Pucci¹, M. Guarna², T. Hadjistilianou³, G. Zerega⁴, M. Pierluigi⁴, B. Franco⁵, G. Morgese¹.* 1) Department of Pediatrics, University of Siena, Siena, Italy; 2) Electronic Microscopy Unit for Genetics, Department of Biomedical Science, University of Siena, Siena, Italy; 3) Department of Ophthalmology and Neurosurgery, Policlinico Le Scotte, University of Siena, Siena, Italy; 4) Human Genetics Laboratory, Galliera Hospital, Genoa, Italy; 5) Telethon Institute of Genetics and Medicine (TIGEM), San Raffaele Biomedical Science Park, Milan, Italy.

We report on a two-year-old girl with a de novo mutation [45,XX,der(5),t(5;14) (pter;q11.2)] and a neuroectodermal disorder, resembling the phenotype of microphthalmia with linear skin lesion (MLS). She had corpus callosum agenesis, multiple cysts (cerebral and cardiac), subtle ocular abnormalities and at least two different skin defects, strongly indicating neuroectodermal involvement: first, a neuromuscular choristoma (hamartoma); second, an eccrine hamartoma. Fluorescent in situ hybridization experiments with different single-locus probes were performed, to determine the extent of chromosome involvement in the translocation [45,XX,der(5),t(5;14) (pter;q11.2)]. The results showed that chromosome 5 has a very small deletion, confined to a region composed of repetitive sequences. By contrast, the q arm of chromosome 14 seems much more important, and is involved in the rearrangement, with partial monosomy spanning from the centromere to the D14S72 and D14S261 loci. The extension of the deletion region of chromosome 14 is approximately 16 cM. To our knowledge, this is the shortest reported deletion involving the chromosome 14q11.2 region to be associated with a developmental disorder resulting in variable eye, skin and brain anomalies (e.g., corpus callosum agenesis), similar to the MLS phenotype. We suggest that a new syndrome, mimicking the MLS phenotype, could be caused by a de novo autosomal mutation on the distal long arm of the chromosome 14q11.2 region.

Ocular anomalies in 22 Brazilian patients with Williams-Beuren syndrome. *S. Sugayama¹, L. S^{1,2}, D. Bertola¹, L. Albano¹, P. Gerritsen Plaggert¹, S. Bechara², C. Kim¹.* 1) Pediatrics, Inst da Crianca, Sao Paulo, Brazil; 2) Ophthalmology, Hospital das Clinicas, Sao Paulo, Brazil.

Williams-Beuren syndrome (WBS) is a rare contiguous gene deletion syndrome with typical facies, cardiovascular anomalies, mental retardation and a friendly and outgoing personality. The syndrome is caused by a submicroscopic deletion in the chromosome region 7q11.23 which is detectable by FISH analysis in 90-95% of the patients. Main ocular anomalies described in WBS patients are stellate anterior iris stromal pattern, strabismus, hypermetropia and retinal vascular tortuosity. We report on ophthalmological evaluation of 22 patients (13M:9F); all sporadic cases. They presented typical elfin facies, neuropsychomotor delay/mental retardation, skeletal and dental anomalies. Deletion of the elastin gene analysed by FISH was found in sixteen patients (73%). An indirect ophthalmoscopy of the ocular fundus was performed in all patients; a slit-lamp microscopy was done in 20 patients. The age of ophthalmological evaluation ranged between 2y and 17y (mean 8,7y). The following ocular anomalies were observed: strabismus (41%), punctate opacities in the lens (33%), stellate iris pattern (28%), retinal vascular tortuosity (29%) and optical nerve hypoplasia (9%). So far, the punctate lens opacities observed by slit-lamp have not been previously described in WBS patients. Half of the patients who presented these punctate opacities have hypercalcyuria and two others have serum calcium in the high normal range. Once hypercalcemia is a known cause of lens opacities, this metabolic disturb could be responsible for this finding in WBS patients. Ocular anomalies in WBS children may have implications in cognitive development. Thus, we recommend a regular ophthalmological examination for all children with WBS.

A case of multiple congenital anomalies due to an unbalanced intrachromosomal rearrangement of chromosome 7. V.M. Rose, P.L. Plotner. Div. Medical Genetics, Dept. Pediatrics, The University of Texas Medical School, Houston, TX.

Duplication of 7p terminus and deletion of 7q terminus are both rare events with about 20 described patients in the literature. Characteristic features of individuals with 7p terminal duplications include psychomotor retardation, dolichocephaly or microbrachycephaly, large fontanels, hypertelorism, large apparently low-set ears, micrognathia, hyperextensible joints subject to dislocation, joint contractures and a high rate of cardiac septal defects. Analysis of reported breakpoints suggests that the critical region for this phenotype is 7p15® pter.

Characteristic features of individuals with 7q terminal deletions include developmental and growth retardation, unusual facies, cleft lip/palate, genital anomalies (males), abnormal EEG, microcephaly and eye defects. Holoprosencephaly has been variably reported. Analysis of reported breakpoints suggests that the critical region for this phenotype is 7q35® qter.

We report a case of an infant with multiple congenital anomalies and a 7p terminal duplication and a 7q terminal deletion. The proband is the product of her nonconsanguineous parents' second pregnancy. The course of the pregnancy was unremarkable. At birth, the infant was noted to have multiple congenital anomalies including: small for gestational age, microcephaly, dislocated hips bilaterally, and right club foot. Echocardiogram revealed thickened, dysplastic heart valves. An initial finding of hydronephrosis resolved over a 2 month course. Karyotypic analysis showed an unbalanced intrachromosomal rearrangement of one chromosome 7. This rearrangement resulted in a deletion of band 7q36 to qter and a duplication of band 7p21.3 to pter: 46,XX,der(7)del(7)(q36® qter)dup(7)(p21.3® pter). Parental chromosome analyses were normal.

The patient displays distinct characteristics of both 7p terminal duplication and 7q terminal deletion syndromes. We propose that the critical regions of each syndrome can be further refined based on the patient's clinical and karyotypic information. Also, further delineation of the clinical course will be obtained by continued observation.

Autoimmune disorders in velo-cardio-facial syndrome. . *E.K. Schorry, H.M. Saal, M.H. Passo, H.I. Brunner.*
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Autoimmune disorders are becoming a well-recognized manifestation of deletion 22q11.2 (velo-cardio-facial syndrome, VCFS). We present two patients with VCFS with multiple autoimmune disorders. Case 1 was referred to genetics at age 15 years for evaluation of possible VCFS. She had a history of neonatal seizures due to hypocalcemia, submucous cleft palate, and developmental handicap. She had multiple hospital admissions beginning at age 3 years for thrombocytopenia, hemolytic anemias, and autoimmune neutropenia. At age 13 she presented with erosive polyarticular arthritis. VCFS was diagnosed at age 15.

Case 2, an 8 y.o. white male, had a history of laryngeal web, right sided aortic arch, and speech delay. Autoimmune manifestations included thrombocytopenia and hemolytic anemia. VCFS was diagnosed at age 8 years. Some of the facial features of VCFS were obscured by prednisone therapy in this patient.

We conclude that a wide variety of autoimmune disorders are possible complications of VCFS. The etiology is unknown, but may be related to T-cell dysfunction. Typical facial features of VCFS may be distorted by JRA or steroid treatment. Clinicians should keep a high index of suspicion of VCFS in patients with autoimmune disorders. Likewise, clinicians caring for patients with VCFS should be aware of their increased risk for autoimmune dysfunction.

Turner Syndrome: A genetic model for Attention-Deficit/Hyperactivity Disorder. *H.F. Russell¹, T.J. Power¹, L.E. Mitchell¹, P.M. Robins², J.L. Ross³, M. Muenke⁴.* 1) The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA; 2) duPont Hospital for Children, Wilmington, DE; 3) Thomas Jefferson University, Philadelphia, PA; 4) Medical Genetics Branch, National Human Genome Research Institute, Washington DC.

Girls with Turner Syndrome (TS) have previously been found to exhibit increased levels of hyperactivity and inattention. However, no studies have assessed whether these individuals meet strict (DSM-IV) criteria for Attention-Deficit/Hyperactivity Disorder (ADHD). The present study evaluated 52 girls with TS between the ages of 7 and 16 years in order to determine the prevalence of ADHD in this group. Cognitive ability and academic achievement were assessed, in addition to parent and teacher behavioral rating scales, in order to make the diagnosis of ADHD. Preliminary analysis of the data on the first 45 subjects yields a significantly higher Verbal IQ (101.2 +/- 12.5) than Performance IQ (85.8 +/- 13.0) with a Full Scale IQ of 93.1 +/- 12.8. This split between verbal and non-verbal skills has been previously demonstrated in girls with TS.

The behavioral data demonstrate more than a 15 fold increase ($p < .01$) in the prevalence of ADHD in girls with TS (24%) compared to girls in the general population (1.3%) and a 4.4% increase ($p < .05$) when compared to the prevalence of ADHD in boys and girls in the general population (5%). Of the TS subjects with ADHD, the Predominantly Inattentive Type ($n=4$) and the Predominantly Hyperactive/Impulsive Type ($n=4$) were most common, followed by the Combined Type ($n=3$) suggesting an alteration in the pattern of subtypes present in girls in the general population.

Molecular studies show no evidence of a relationship between parent of origin of the intact X chromosome and ADHD status ($p=1.0$). In addition, these data demonstrate no relationship between karyotype (i.e., XO vs. mosaic) and ADHD status ($p=1.0$). Overall, these data support the notion of a gene(s) on the X chromosome predisposing to ADHD. This gene(s) might be identified using detailed linkage studies.

Osteoporosis in neurofibromatosis type 1 (NF1). *M. Poyhonen*¹, *J. Heikkinen*², *K. Väänänen*³, *J. Peltonen*^{4,5}. 1) Dept Medical Genetics, Family Federation of Finland, Helsinki, Finland; 2) Osteoporosis Clinic, Oulu Deaconess Institute, Oulu, Finland; 3) Department of Anatomy, University of Turku, Turku, Finland; 4) Department of Anatomy and Cell Biology, University of Oulu, Oulu, Finland; 5) Department of Medical Biochemistry, University of Turku, Turku, Finland.

Skeletal changes are common and well-known in neurofibromatosis type 1 (NF1). The most frequent findings include scoliosis in about 10 % of NF1 patients and congenital bowing and thinning of the long bones with or without pseudarthrosis in about 3 % of the cases. Other skeletal abnormalities consist of erosive defects due to the presence of neurofibromas contiguous to bone, disorders of growth, intra-osseous cystic lesions, and congenital anomalies of the skeleton such as sphenoid bone wing dysplasia. The pathophysiology of these changes in NF1 are still mostly unknown and osteomalacia has been reported only in a few cases. In spite of the actively ongoing research into NF1, there are still only a few reports, mainly case reports, that have investigated the role of osteoporosis in the disease. We report a systematic study on bone density evaluated by LUNAR-DPX (DEXA) scan in 35 NF1 patients. The NF1 diagnosis was confirmed in all cases by the NIH consensus criteria. Of the patients 21 were male and 14 female. The age distribution ranged from 11 to 73 years (mean 37 years). In 26 adults (over 20 years, 14 male and 12 female) the bone density evaluated by scan revealed osteoporosis in 13/26 (50 %). The osteoporosis was mild in 10 cases (T-score -1.0 - -2.4) and moderately severe in 3 cases (T-score -2.5 or under). The bone density varied in different regions being in all cases lowest in the ribs and highest in the head. Also the bone mineral content (BMC) gave different results at different sides of the same region. The total bone calcium in adults varied in males from 799 g to 1416 g and in females from 582 g to 1025 g. The risk of developing osteoporosis may be increased in patients with NF1, especially with young adults this risk needs to be recognized, and bone density should be evaluated in the potentially at-risk patients.

New evidence for autosomal recessive mode of inheritance in hypomandibular faciocranial dysostosis. C.

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Hypomandibular faciocranial dysostosis is a rare disorder, presenting with craniosynostosis, prominent eyes, deficient midface and zygomatic arches, short nose with anteverted nares, protruding lower face, major microstomia, persistent buccopharyngeal membrane and severe mandibular hypoplasia. Three cases have been yet reported and the observation of recurrence in two sibs has suggested an autosomal recessive mode of inheritance. Here, we report on the prenatal diagnosis of an affected female infant born from consanguineous parents. At 31 weeks of gestation, prenatal manifestations included severe microretrognathia and microstomia. The diagnosis of hypomandibular faciocranial dysostosis was suggested. The child was born at 33 weeks of gestation. Birth weight was 2,110 g, length 46 cm and head circumference 33 cm. The child died few minutes after birth of respiratory distress. Autopsy features were down slanting palpebral fissures, deficient midface and zygomatic arches, short nose with anteverted nares, low-set ears, protruding lower face, major microstomia, severe hypoglossia, persistent buccopharyngeal membrane, bifid uvula, laryngeal hypoplasia, median cleft palate and severe mandibular hypoplasia. Examination of the brain was normal. Despite the absence of craniosynostosis the clinical manifestations in the child are highly suggestive of hypomandibular faciocranial dysostosis. The parental consanguinity argues in favor of an autosomal recessive mode of inheritance. Other reports will help to better delineate this rare entity.

Wolf-Hirschhorn Syndrome: developmental outcome and mortality correlate with deletion size. *N.L Shannon¹, R. Regan², E.L. Maltby¹, J. Flint², A.S. Rigby¹, D.M.B. Hall³, O.W.J. Quarrell¹.* 1) Sheffield Children's Hospital, U.K; 2) Wellcome Trust Centre for Human Genetics, Oxford, U.K; 3) Inst. of General Practice, University of Sheffield, U.K.

Wolf-Hirschhorn Syndrome is a well recognised deletion syndrome. Early research described a high mortality and poor developmental outcome irrespective of deletion size but there has never been a large scale study to prove or refute this. The prognosis may need to be revised as children with smaller deletions are now detected and treatment for congenital anomalies has improved. These observations led us to undertake a clinical and epidemiological study. Details of 156 cases were collected from the UK; of these, 108 (69.2%) were *de novo* deletions. The status (alive or deceased) has been confirmed in 143 of which, 96 are alive, 34 deceased and 13 were detected on prenatal testing. Age at death was ascertained in 31 cases. The overall mortality was 22.5% but the data show a significantly higher mortality (48%) in children with deletions proximal to and including p15.2 compared to those with smaller deletions (10%). The relative risk is 8.4 (95% CI=2.5, 28.7). 77.4% of deaths occurred in the first 2 years of life. 66 individuals were visited at home; developmental progress was measured using the Vineland Adaptive Behaviour Score normalised for age. 46 families consented to blood collection for repeat cytogenetic analysis and in addition, 11 PAC FISH probes were developed along 4p to provide an objective measure of deletion size. There was a trend for lower Vineland scores with increasing deletion size ($p=0.08$) after adjusting for age by analysis of covariance. Mean values for small (p16.3-p16.2), medium (p16.1) and large deletions (p15.3 and larger) were 55.0, 48.6 and 45.7 respectively. A separate analysis of FISH data showed a similar trend; larger FISH deletion scores were associated with lower Vineland scores (mean change= -1.2; 95% CI= -2.6, 0.2). Therefore, the regression analysis confirms our clinical impression that children with smaller deletions make significantly better developmental progress. These findings will have important implications when discussing the prognosis with families.

Navajo poikiloderma with cyclic neutropenia in Turkish children, clinical description and exclusion of linkage to the Rothmund Thomson RECQL4 gene. *J.L. Van Hove¹, S. Grünewald², K. Minner², C. Van Geet¹, G. Matthijs².* 1) Dept Pediatrics, Catholic University Leuven, Leuven, Belgium; 2) Center for Human Genetics, Catholic University Leuven, Leuven, Belgium.

Navajo poikiloderma is a unique genodermatosis described in 14 Navajo American Indians consisting of poikiloderma, pachyonychia, neutropenia, and chronic lung disease. We describe two siblings, a girl aged 21 months and a boy of 12 years, out of four children born to consanguineous Turkish parents. They developed a papular erythematous rash on the limbs in infancy, which developed into pronounced teleangiectasis predominantly acral and facial, and not limited to sun-exposed areas as would be seen in Rothmund Thomson syndrome (RTS). Both children have hyperkeratosis under the feet, and pachyonychia of the toes. They have mild dyserythropoiesis and persistent neutropenia of 100-400/microL, which responded with increased neutrophils with infections. They both had hypoglycemia and transient thrombocytopenia in the neonatal period. The boy, born at 34 weeks gestational age, had a grade IV intraventricular bleeding with subsequent hydrocephalus, spastic tetraparesis, and mental retardation. The girl has mild developmental delay. They have very frequent purulent upper respiratory tract infections, otitis media, and bronchopneumonia. Patchy areas of hypercalcification are noted on pelvic X-rays. Mutations in the human helicase gene RECQL4 on chromosome 8q24.3 have been found in RTS patients. Using closely linked polymorphic markers spanning this region, we found that both affected children did not share similar parental chromosomes, excluding linkage of this gene to the Navajo poikiloderma in our patients. This report expands the clinical description of this rare genodermatosis, and includes patients outside the Navajo population. The exclusion of the RECQL4 region adds genetic differences to the clinical distinction from RTS.

A Possible Locus for Setleis Syndrome - A Rare Ectodermal Dysplasia. *K.J. Swanson, X. Li, J. Bergoffen.* Genetics, Kaiser Permanente, San Jose, CA.

Setleis Syndrome is a rare congenital condition with characteristic ectodermal findings of the face. Typical abnormalities include bitemporal scarring resembling forceps marks, periorbital puffiness, wrinkling of the facial skin, upslanting laterally deficient eyebrows, scalp hair and eyelash abnormalities, bulbous nasal tip and thick protruding lips. Growth and psychomotor development are usually not impaired.

Our patient OA is a 6 yo boy with typical facial features seen in Setleis syndrome. In addition, he has short stature, vocal cord paralysis, laryngomalacia, stridor, h/o sleep apnea, atrioseptal deviation, redundant pulmonic valve, GERD, hydroceles, brachydactyly, unusual finger positioning, and severe developmental and attention problems with autistic features.

Peripheral blood chromosome analysis revealed a male karyotype with a de novo interstitial rearrangement within the short arm of chromosome 1. This rearrangement appears to be an inverted duplication in 1p from subbands 1p36.21 to 1p36.13. Fluorescence in situ hybridization (FISH) using DNA probes TEL1p1q (subtelomeric, Cytocell Ltd.) and D1Z2 (1p36.3, Oncor Inc.) revealed normal hybridization patterns and therefore ruled out an unbalanced translocation involving 1pter and another chromosome. FISH analysis with a partial chromosome paint probe for 1p (AI Technology) confirms the 1p origin of the additional segment.

To our knowledge, this is the first report of an isolated 1p duplication involving this region. Halal et al. (1989) reported a newborn with a duplication of 1p31-pter and a deletion of 2q33-qter who presented with microcephaly, cryptorchidism, facial anomalies, and growth and mental retardation but no features of Setleis Syndrome. We propose that the locus for Setleis syndrome is in the region between 1p36.21-1p36.13. The manifestations of Setleis syndrome in our case are likely due to a disruption of the gene by the inversion or a submicroscopic deletion. The additional findings, which are not typically associated with Setleis syndrome, would then be secondary to the partial duplication of chromosome 1p.

Duplication 1q32-q42 syndrome in a child with α -Thalassemia trait. *K.H. Ramesh¹, D. Wei¹, L. Cheng¹, S. Soni², N. Green², P. Levy², L.A. Cannizzaro¹.* 1) Dept Pathology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; 2) Dept. Pediatrics, Montefiore Medical Center, Bronx, NY.

Phenotypic/genotypic correlation of the duplication 1q syndrome is not yet clearly established due to wide variations in presenting clinical features. The severity of mental and growth retardation along with other dysmorphic features depend on the extent of the duplicated segment. The larger the duplication, the more severe are the associated malformations. We report a 14 year old female of Cambodian ethnicity with a history of development and speech delay, as well as, persistent anemia. Physical examination revealed short stature, severe developmental delay, mental retardation, with minimal dysmorphic features. These include height, weight and head circumference being lower than the 5th percentile, brachycephaly of the head, epicanthal folds, slightly flattened upturned nares, and slight webbing of the neck. Her heart had a grade II/VI vibratory systolic ejection murmur. Genitalia were normal female, but with delayed puberty. Exam of extremities revealed clinobrachydactyly of the 5th digits of both hands and clinodactyly of the 5th digits of both feet. Cytogenetic analysis detected a 46,XX,dup(1)(q32q42) karyotype in all cells and was confirmed by whole chromosome painting analyses. The mother's and (half) sister's karyotypes were normal. The father was unavailable for cytogenetic testing. The proband was also positive for α -thalassemia trait. Southern blot analyses confirmed this, showing deletions of two of the α -globin genes, a condition that is common among Southeast Asians, and its occurrence in this proband with the 1q syndrome may be purely co-incidental. The clinical features present in this proband show some similarities as well as differences when compared with other documented cases of 1q duplication of the q32-q42 segment. This suggests that the various breakpoints involved, may be critical in the manifestation of some of the features in this syndrome. Interestingly, the Coagulation Factor XIII B (F13B) gene has been mapped to the 1q31-q32 region. It would be worthwhile to determine if this gene is duplicated or deleted in this particular patient.

Triophtalmia and Down syndrome: a previously unreported association. *C. Perandones^{1, 2}, M. Segovia¹, J. Golberg², O.H. Pivetta¹, R.D. Bennun².* 1) centro Nacional de Genetica Medica. Buenos Aires, Arentina; 2) Piel, Cirugia Plastica Infantil, Avellaneda, Argentina.

The first report of triophtalmia was performed in 1995 by Cohen and Stelnicki. The patient presented a duplication of the left globe and left cerebral hemisphere. They termed this rare anomaly triopia. Another case was documented by Tayel in 1998 in a boy with an unusual phenotype including facial clefting. We present the previously unreported association of triophtalmia and Down syndrome (DS). A five year-old girl was referred for consultation due to the presence of unilateral exophtalmia. She has been diagnosed as having Down syndrome soon after birth. Her karyotype was 47,XX, + 21. The pregnancy was uneventful and there was no history of drug intake or exposure to irradiation, infection or teratogens during pregnancy. Preoperative CT scan revealed the presence of a solid retro-ocular mass. The tumor was excised by an extracranial approach using a lateral external orbitotomy. Histological studies confirmed the presence of ocular structures. Taking into account the rate of trisomy 21 (1/ 645) and the fact that triophtalmia was only previously reported twice, the probability of concurrence by chance in the same patient of triopia and DS is very low. Thus, a causal relationship between both conditions must be suspected. Shapiro (1983) has suggested that aneuploidy may lead to magnified responses to disrupting agents. So, trisomy 21 may increase susceptibility in the embryo to develop triophtalmia in the presence of certain environmental factors. However, in our case, this factor did not exist and no other potential teratogenic factors could be identified. Several genes involved in the regulation of eye development have now been identified including the mouse Pax 6 gene, which is believed to play a master role triggering the development of eye components. However, none of these genes has been mapped to chromosome 21. The aim of this report is to describe a previously unreported association and to discuss embriological and molecular mechanisms potentially involved in its origin.

Post-zygotic origin of a biparental 13;13 Robertsonian translocation in the mother of a fetus with trisomy 13.

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A 29-year-old primigravida was referred for further evaluation of multiple congenital anomalies identified in her fetus by a screening obstetric ultrasound. A level II ultrasound showed a single vertex intrauterine pregnancy with a mean gestational age of 29.3 weeks; 2.8 weeks behind her established dating by LMP, as well as the following fetal abnormalities: mid-line cleft lip, partial lobar holoprosencephaly absent anterior horns with dilated posterior horns and lateral ventricles, a prominent thalamus, hypotelorism, a flat sacral neural tube defect and abnormal positioning of the toes. Due to the fetal position, the heart could not be evaluated. Amniocentesis revealed a male fetus with trisomy 13 associated with a derivative chromosome 13: 46,XY,der(13;13)+13. Paternal chromosome studies were normal, 46,XY, whereas, the maternal karyotype was 45,XX,der(13;13). Subsequent cytogenetic studies demonstrate that the der(13;13) was dicentric and the revised karyotype was reported as 45,XX,dic(13;13)(p11.1;p11.1). To determine the parental origin of the maternal 13;13 chromosome rearrangement, the mother and her parents were genotyped using a panel of seven microsatellite markers from chromosome 13. Five of the seven markers were fully informative and showed biparental inheritance of the alleles, demonstrating that the maternal 13;13 was a true Robertsonian translocation and not an isochromosome. Several previous studies have addressed the parental origin of de novo 13;13 chromosome rearrangements. Most homologous rearrangements of chromosome 13 studied have been determined to be isochromosomes, whereas only a few were found to be true Robertsonian translocations. Our case of a balanced 13;13 translocation confirms that Robertsonian chromosomes may originate at conception or postzygotically.

Trisomy of the short arm of chromosome 5 due to a de novo inversion, duplication (5)(p13-p15.3). *A. Gonzalez-del Angel, M. Cervera, S. Sanchez, B. Molina, M.A. Alcantara, A. Carnevale, V. del Castillo.* Dpto. Genetica Humana, Instituto Nacional de Pediatria, Mexico.

Partial trisomies of the short arm of chromosome 5 are uncommon. It has been shown that patients with duplication distal to 5p13.3 have mild and relatively different phenotypes as compared to patients with complete duplications and proximal duplications that include band 5p13, suggesting that the critical region for 5p trisomy syndrome lies between 5p10 and 5p13.1. We report a 5 year-old Mexican girl who developed severe mental retardation and generalized tonic clonic seizures at one year-aged. She had cranium with tendency to turriccephaly, upslanted palpebral fissures, bilateral inner epicanthal folds, deep nasal root, and malformed ears with posterior rotation. The cytogenetic study with G bands in lymphocytes from peripheral blood showed a 46,XX karyotype, in which one of the chromosome 5 had an extra segment on the short arm in all the analyzed cells, this suggested a 5p duplication. We performed FISH technique to confirm the diagnosis. The hybridization with a coatosome 5 total chromosome probe, revealed that the additional segment involved the same chromosome. In addition, with the Cri-du-chat 5p15 unique sequence probe, we observed two fluorescent signals on the short arm of the chromosome. We concluded that our patient had a duplication and a inversion of 5p: 46,XX,inv,dup(5)(p13p15.3 :: p15.3 qter). Both parents had normal karyotypes. As far as we know, only one case has been reported in the literature by Kleczkowska et al. (1987) who also had the same cytogenetic rearrangement as our patient but with a different phenotype. Since they only performed conventional cytogenetics, the differences in the phenotypes between both patients could be explained by the presence of other chromosome regions involved in the rearrangement found in the patient described previously by Kleczkowska et al. This case is a clear example that molecular cytogenetics is a complementary tool of conventional cytogenetic analysis. The combined use of these methods allows a precise diagnosis and an accurate genetic counseling as is illustrated in the present case.

Partial trisomy 4q and monosomy 18p resulting from a de novo unbalanced translocation. *P.L. Devers, A. Asamoah, K. Zubrickas, D.L. Van Dyke.* Department of Medical Genetics, Henry Ford Hospital, Detroit, MI.

We present a case of a male infant with a de novo translocation involving chromosomes 4q and 18p and resulting in duplication 4q31-qter and deletion 18p11.2-pter. The pregnancy was complicated by premature rupture of the membranes and delivery by c-section because of heart decelerations. Birth weight was 1473 g and birth length was 42 cm. Chromosome analysis was obtained because of dysmorphic facies and a single umbilical artery. The neonatal course was significant for jaundice, temperature instability, ventricular septal defect and patent foramen ovale. Physical examination at 2 months and 6 months revealed length, weight and head circumference below the 5th percentile, prematurely aged facies, retrognathia, carp mouth, broad alveolar ridge with gingival hyperplasia, excess skin around the base of the penis, and hypertonia. Development at 6 months was delayed. Surgical correction was necessary for malrotation of the bowel. Immunoglobulin levels were normal. The G-banded karyotype from peripheral blood lymphocytes culture was 46,XY, add(18)(p11.2).

FISH analysis using a chromosome 4 painting probe (Oncor, Inc.) identified the additional material to be derived from chromosome 4. The karyotype was interpreted as 46,XY,der(18)t(4;18)(q31;p11.2). Parental karyotypes were normal. Features of trisomy 4q syndrome include growth retardation, mental retardation, microcephaly, congenital heart defects, and dysmorphic facies (epicanthal folds, low set ears, micrognathia, carp mouth). Features of deletion of the short arm of chromosome 18 include low birth weight, short stature, mental retardation, carp mouth, ptosis, and IgA deficiencies. The clinical features of the present case are similar to those reported previously with each of these two chromosome abnormalities. To our knowledge, malrotation of the bowel has not previously been reported in association with either duplication 4q or deletion 18p.

Ring chromosome 22: Physical and developmental profile in 8 patients. *G.A. Anadiotis¹, L. Celle¹, S. Saitta¹, I.D. Krantz¹, E.M. Kaye¹, N.B. Spinner¹, B.S. Emanuel¹, P.P. Wang², E.H. Zackai¹.* 1) Division of Human Genetics and Molecular Biology; 2) Division of Child Development and Rehabilitation, The Children's Hospital of Philadelphia.

We have analyzed the phenotype of eight patients with a constitutional non-mosaic ring chromosome 22. Five females and three males ranging from 17 months to 6.5 years underwent full physical and developmental evaluations. At birth all eight patients had normal height, weight and head circumference. At the time of evaluation, all had normal physical growth, except for 1 female with head circumference less than the fifth percentile. Two of eight patients had multi-systemic involvement including TAPVR, VUR, umbilical hernia and tracheomalacia (patient 1); multicystic kidney and polycystic ovaries (patient 3). Dysmorphia if present, was minor. Seven patients ranging between 17 months and 36 months of age had gross motor delay, three of which were in the severe category. Language was delayed in all seven and worse than motor skills in four. The eighth child was aged 6.5 years and had an IQ of 71 (Stanford-Binet). This further emphasizes the need for chromosome studies in non-dysmorphic children with developmental delay.

Natural history of inv dup (8p): Clinical and cytogenetic findings in six cases. *K. Kurosawa¹, H. Kawame², Y. Ochiai², M. Matsuo¹, K. Imaizumi¹, Y. Kuroki¹.* 1) Kanagawa Children's Med Ctr, Yokohama, Japan; 2) Tokyo Metropolitan Kita Med Rehab Ctr, Tokyo, Japan.

Inverted duplication of short arm of chromosome 8 (inv dup (8p)) is one of the most common unbalanced structural abnormalities and is associated with a well-delineated clinical picture. The duplicated region includes band 8p11.2 to 8p23. We report on the natural history of 6 Japanese cases with inv dup (8p) according to the medical records. Cytogenetic abnormalities of chromosome 8 were noted in all patients, but FISH with whole chromosome painting of chromosome 8 and probe D8S596 (ONCOR) were performed in four. Parental origin of inv dup (8p) was analyzed in 3 families with polymorphic microsatellite markers. Sex ratio (M:F) of 6 cases are 1:5. The ages ranged from 3 years and 6 months to 10 years. The parental ages at birth were no different from those of general population. Seizures and feeding difficulties were the most important problems in early infant period. However, seizure control with anti-convulsant agents provided generally good prognosis on this neurological problem. Agenesis or hypoplasia of corpus callosum was a common feature of central nervous systems. The developmental milestone was severely retarded and the degree of retardation was increasing for age over infant period. Final scores of developmental quotient of the cases were less than 10 except for case 2. Although no patient attained to walking without support and speaking a meaning word, eye contact and simple emotional expression was possible. In childhood, the stable daily life was attained in most cases. Attending on the special school was possible in three. Few life-threatening problems were held in most cases over infant periods. Maternal origin of de novo inv dup (8p) was revealed in one of 3 pedigrees analyzed with microsatellite marker D8S264, which is located at 0.7cM from 8p terminal. These information is of great importance for understanding of the natural history of inv dup (8p) and useful for caring the patients with the disorder.

Terminal deletion of chromosome 1p36: New insights into clinical features and medical management. *J.A. Johnson, R.I. Blough, C.H. Jayne, E.K. Schorry, R.J. Hopkin, H.M. Saal.* Division of Human Genetics, Children's Hospital Medical Center, Cincinnati, OH.

A very common but under recognized chromosome deletion syndrome is that of deletion 1p36. In our institution we have identified this deletion in 9 patients over the past 5 years from a total of 4500 cytogenetics samples. All deletions were apparently de novo. Most patients were identified because they presented with developmental delays and congenital malformations. The facial dysmorphism was consistent among most patients with brachycephaly, prominent forehead, micrognathia, small mouth and short palpebral fissures. Other consistent features were seizures seen in 6 patients, hypotonia, developmental disabilities, and severe verbal apraxia. Genital anomalies were frequent in males. One patient had a cleft lip with cleft palate and one patient had Pierre Robin sequence with a cleft palate. A very common problem in infants was failure to thrive despite adequate caloric intake. In our institution this is the second most common chromosome deletion syndrome, after velocardiofacial syndrome. The level of suspicion for this condition should be raised in the presence of the typical dysmorphic features, seizures, severe verbal apraxia, and failure to thrive.

Two cases of partial trisomy 10q and monosomy 7q with features similar to Cornelia de Lange syndrome, including split hand/split foot deformity. *S. Randall-Pinto¹, G. Johnson¹, V. Lopes¹, I. Teshima², P. Wyatt¹.* 1) Dept. of Genetics, North York General Hosp., Toronto, ON, Canada; 2) Hospital for Sick Children, Toronto, ON, Canada.

A 38 year-old woman presented for advanced maternal age counselling. Family history revealed a child that died at 2 days of age of Cornelia de Lange syndrome. Parental chromosomes later ordered for pregnancy losses demonstrated that the father was a balanced translocation carrier; 46,XY,t(7:10)(q32;q24).

The couple elected to have amniocentesis at which time holoprosencephaly was detected. The pregnancy was terminated and chromosome analysis revealed a 46,XY,der(7)t(7:10)(q32;q24) karyotype. Pathology assessment revealed microcephaly, hypotelorism, central proboscis, macrognathia, short neck, pterygia, bilateral absent forearm bones, bilateral claw hands with two digits each, imperforate anus, unilobar right lung, holoprosencephaly, hypoplastic right heart, and pulmonary artery stenosis.

Records were obtained for the earlier neonatal death. Multiple congenital anomalies similar to those previously described were noted including a short webbed neck, arthrogryposis, and absent ulna with a single digit. Although chromosomes were initially reported as normal, a subtle imbalance was detected after reviewing both the father's chromosomes and those from the terminated pregnancy.

To the best of our knowledge, this chromosome abnormality has not previously been reported. Further, the presence of split hand/split foot deformity in each of these cases supports the hypothesis that a gene involved in limb development is localized to chromosome 10q24¹. We therefore propose the addition of split hand/split foot deformity to the clinical spectrum of abnormalities associated with distal 10q trisomy syndrome and recommend careful investigation of chromosome 10q24 in patients with apparent Cornelia de Lange syndrome including split hand/split foot deformity.

1)Nunes ME, et al., Hum. Mol. Genet. 4:2165, 1995.

Cytogenetic(Somatic and Germinative)and Molecular(AZF,CFTR and AR genes)evaluation in Male Infertility.

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Approximately 10% of phenotypically normal men are infertile, with 30-40% of them classified as idiopathic. The genetic factors involved in human male infertility are chromosomal(classical somatic chromosomal aberrations and germinative meiotic anomalies),mutations of the CFTR and AR genes and, microdeletions of AZFs genes(Yq11 region).In the present study we performed a clinical,cytogenetic(100 metaphases from blood lymphocytes and a meiotic study from testicular biopsy)and molecular(27 primers for AZFa,b and,c by PCR multiplex;24 mutations of the CFTR gene;and,mutation and deletion study of the AR gene)evaluation of a sample of 70 infertile men with the major objective of determining the frequencies of this genetic factors in brazilians patients.The results are:30%(21/70)of the infertile men presented genetic etiologies; 14.3%(10/70)presented environmental etiologies, and 39(55.7%)remain idiopathic after the complete evaluation.Among the genetic etiologies we detected 7.1%(5/70)with classical somatic chromosome aberrations(4 Klinefelter syndrome and one with a 13/14 robertsonian balanced translocation);7.1%(5/70)presented meiotic(asynapsis)anomalies;4.3%(3/70)presented microdeletions of the AZFc gene causing disruption of the DAZ gene; and, 2.8%(2/70)were heterozygotes for mutation of the CFTR gene(all with CAVD).The importance of these results are discussed.

Deletion of SHOX gene without short stature, Turner skeletal features or Leri-Weill syndrome. *H. Thiele, C. Baldermann, I. Hansmann.* Institut fuer Humangenetik, Martin-Luther Universitaet, Halle, Germany.

Haploinsufficiency of the SHOX gene has been shown to cause not only short stature but also Turner skeletal features, such as short 4th metacarpals and cubitus valgus as well as Leri-Weill syndrome (LWS). We report on a 20-year-old woman with hemizygosity for a Xp22 segment including the SHOX gene in about 75% of her lymphocytes without short stature, Turner skeletal features or LWS. The 20-year-old female was referred for genetic counselling and cytogenetic diagnosis due to primary amenorrhoea. At 5 months of age a muscular torticollis and a talipes calcaneus was observed. At the age of 1 year she presented with a severe progressive idiopathic thoracic scoliosis. Later on a displaced right kidney was observed. At referral to genetic counselling her height was 162 cm with a weight of only 46.1 kg. Hearing and smelling were normal and there were no signs for dental anomalies or ichthyosis. Family history is reported to be normal without any sign of skeletal defects, scoliosis or infertility. Chromosome analysis was performed on G-banded metaphases of cultured lymphocytes at 500 band stage. Due to slightly altered band staining at Xp22 in several but not all metaphases FISH analysis was performed using cosmid 34F5 containing the SHOX gene, kindly provided by G. Rappold (Heidelberg). FISH signals for SHOX were observed at the distal end of the short arm of both X chromosomes in 7/30 metaphases (23%). In 23/30 metaphases (77%) only one X chromosome revealed signals at the distal p-arm. A Y chromosome paint revealed characteristic PAR1 signals in 5/20 metaphases (25%) at the distal end of the short arm of both X chromosomes and on only one Xp-chromosome in 15/20 metaphases (75%) thus confirming the Xp-deletion of SHOX in the majority of cells. The karyotype therefore is: 46,XX/46,X,del(X)(p22). Mapping of the breakpoints within Xp22 is in progress using probes flanking the SHOX gene.

Are the phenotypic features of a large interstitial deletion 11q14.2-11q23.1 distinct from Jacobsen Syndrome (terminal deletion 11q)? *C. Theda*^{1,2,3}, *E.M. Garcia Soto*^{1,2}, *C.B. Cargile*^{1,2}, *N. Hendi*³, *M.K. Miller*^{1,3}. 1) Johns Hopkins University School of Medicine, Baltimore, MD; 2) Kennedy Krieger Institute, Baltimore, MD; 3) Frederick Memorial Hospital, Frederick, MD.

The majority of patients with deletions of chromosome 11q reported in the literature have terminal deletions distal to 11q23. It has been postulated that band 11q24.1 is the critical region for the phenotype - which is also referred to as Jacobsen syndrome. This phenotype includes growth and mental retardation, initial hypotonia with later spasticity, trigonocephaly, eye and midface abnormalities, carp shaped mouth, cleft lip and palate, congenital heart defects, and thrombocytopenia.

Our review of the literature identified only two reports of patients with interstitial deletions of chromosome 11q. Deleted regions were 11q21-11q23 and 11q22.3-11q23.2. The reported features of the patients that appeared to be distinct from Jacobsen syndrome were Pierre Robin sequence and Ebstein anomaly of the heart.

We report here a female patient with a 46,XX,del(11)(q14.2q23.1) karyotype. The proband was born vaginally at 38 wks with a birth weight of 2655 g. The infant appeared floppy. Dysmorphic features including depressed nasal bridge, small nose, anteverted nares and significant micrognathia were noted. The fifth toes were both displaced dorsally and overlapped the fourth toes. Further evaluation revealed high but intact palate, cardiac murmur due to peripheral pulmonary stenosis and patent foramen ovale, no thrombocytopenia. Her postnatal course was dominated by feeding difficulties due to the micrognathia and extremely poor suck-swallow coordination. The initial hypotonia resolved and increasing muscle tone and spasticity developed.

A detailed review of the phenotypic features of our patient with a large and previously not described 11q deletion in comparison with the two reported cases of smaller interstitial deletions and patients with Jacobsen syndrome will be presented.

FISH and PCR analysis in three patients with 45X/46X,mar(Y) Karyotype: Clinical and pathologic spectrum. *F. Alvarez, M. Soto, M. Prieto, L. Borjas, A. Rojas-Atencio, J. Cañizalez, Z. Alvarez.* Unidad de Genetica Medica, Universidad del Zulia, Maracaibo, Zulia, Venezuela.

Alterations of sexual differentiation occur as small structurally abnormal sexual chromosome (marker chromosome). We describe the clinical and molecular characterization of three patients with 45X/46X, mar (y) Karyotypes. The patients, two males and one female (ages 2-26 years) had varying degrees of impairment of sexual differentiation, with or without testis formation. One patient (reared as female) had a phenotype far more severely affected than expected in Turner syndrome (mental retardation, growth delayed and an unusual facial appearance) with streak gonad bilaterally. The second patient (12 years old) had ambiguous genitalia with a dysgenetic testis with a contralateral streak gonad. And another patient (26 years old) had bilateral dysgenetic testes (dysgenetic male pseudohermaphroditism). All patients had 45X/46,X,mar(Y). The ratio of the two stemlines was varying between the patients and between different tissues. The marker chromosomes were identified as Y chromosome, using FISH and PCR based analysis to detect Y specific sequence. The marker chromosome was deleted proximal and distal part of the normal Y chromosome, but the SRY gene was present in all patients and all tissues. The extend of male or female differentiation in these patients depends in part on the prevalence, time occurrence, and distribution of the 45,X line and possibly in part on the alteration of other genes (non-SRY) involved in sex differentiation.

Mother to Daughter Transmission of Silver-Russell Syndrome. *C.H. Jones, S. Nourbash.* Dept Pediatrics, Section Gen, Loyola Univ, Maywood, IL.60153.

Silver-Russell syndrome is of unknown etiology. Clinical features include intrauterine growth retardation, postnatal growth retardation and characteristic dysmorphic features. These characteristics include triangular face, small pointed chin, asymmetry, delayed closure of the anterior fontanelle and fifth finger clinodactyly. Other features include mental retardation and developmental delay. We are reporting the case of a mother and daughter both affected with Silver-Russell syndrome (SRS). SC was evaluated at 17 months for failure to thrive and developmental delay. She was noted to have delayed growth parameters. Her weight was 7.82kg (<5 percentile), length 74.5cm (<5 percentile) and head circumference of 43.5cm (5 percentile). She had asymmetry of the face and chest with the left being more prominent than the right. The left leg was 3 cm longer than the right. She had fifth finger clinodactyly bilaterally. Chromosomal analysis was normal (46,XX). SC's mother JC was 19 years of age. She was 60cm tall. She has slight facial asymmetry, limb asymmetry and fifth finger clinodactyly. Her chromosome analysis was also normal (46,XX). Both SC and her mother were diagnosed with Silver-Russell syndrome. Uniparental disomy of chromosome 7 has been described as cause of SRS syndrome in approximately 10 percent of cases. There has also been 17 families described with vertical transmission of SRS in the literature. We report another case of mother to daughter transmission of SRS. This report further demonstrates a possible dominant mode of transmission in addition to variable penetrance with the daughter more affected than the mother.

Clinical, cytogenetical and molecular studies of the Angelman syndrome in 62 Brazilian patients. *G.A. Molfetta¹, V.F. Ferraz¹, W.A. Silva-Jr², J.M. Pina-Neto¹*. 1) Dept Genetics, Sch Medicine, Ribeirao Preto - USP, Sao Paulo, Brazil; 2) Molecular Biology Unit, Fundacao Hemocentro, Ribeirao Preto - USP, Sao Paulo.

The Angelman syndrome (AS) is clinically characterized by central congenital hypotonia, delayed neurological and psychomotor development, severe mental deficiency, absence of speech. Neurologically, the patients have seizures, ataxia and unusual electroencephalogram. The AS is associated with genetic defects in the 15q11-13 region of the maternal chromosome 15. Different genetic mechanisms lead to the onset of AS: 65-70%; of the cases involve de novo deletions in the maternal 15q11-13 region; 3-5%; are uniparental paternal disomy; 7-9%; are mutations in the imprinting center, 4-6%; are mutations in the UBE3A gene and 10-14%; present biparental inheritance. In order to establish the diagnosis of the AS, clinical, cytogenetical and molecular evaluations were performed in 62 patients, in whom the AS diagnosis was suspected. The clinical evaluation established the most frequent phenotypic characteristics in the affected individuals. The cytogenetic investigation was performed to detect chromosomal rearrangements using the GTG, CBG and Ag-NOR banding techniques. The molecular approach was based in the methylation analysis of the 15q11-13 region and segregation of the (CA)_n repeats polymorphism analysis using primers in the 15q11-13 region and in 15qter region. The molecular approach was performed to confirm the diagnosis and determine the genetic cause of AS. Genetic counseling was also offered to the families. The cytogenetical analysis detected two cases with chromosomal abnormality, a pericentric inversion 46, XX, inv(15p;15q) in both of them. All the other cases were chromosomally normal (46,XY). The molecular analysis confirmed 9 cases of AS caused by de novo maternal deletion, 2 cases with mutation in the imprinting center, no cases of paternal disomy and 27 cases showed biparental inheritance. In other 22 cases with biparental inheritance, the diagnosis of AS was excluded by clinical criteria. This method of investigation has proved to be efficient for evaluation of patients under suspect of having AS. Financial Support: FAPESP (process No 98/02378-9).

Update on the clinical features and natural history of Wolf-Hirschhorn syndrome (WHS): Experience with 48

cases. *A. Battaglia*^{1,2}, *J.C. Carey*². 1) Inst Child Neurology & Psych, Stella Maris Inst/Univ Pisa, Pisa, Italy; 2) Dept Peds/Div Med Genet., Univ of Utah Health Sciences Ctr., Salt Lake City, UT.

The knowledge of the natural history of a clinical condition is of the utmost importance for many reasons. To help delineate more accurately the natural history of WHS and to obtain better information to answer parents' questions in a clinical setting, we decided to develop an detailed questionnaire, dealing with the clinical manifestations of the syndrome and with the psychosocial development. The questionnaires were sent to the families of children with WHS, through their national support groups in the Italy and USA. Overall there were data on 48 patients (32 females, 16 males). 35 cases were detected by standard cytogenetics, while the remaining 13 required FISH. 46/48 (95.8%) had a seizure disorder; 21/48 (43.7%) underwent gastrostomy due to feeding difficulties; 24/48 (50%) had heart lesions; 18/48 (37.5%) had oral facial clefts; and 5 (10.4%) of them had sensorineural deafness; 13/48 (27%) had genitourinary tract defects. Of note, only 32/48 (66.6%) had severe/profound developmental retardation; while retardation was moderate in 12/48 (25%), and mild in 4/48 (8.3%). This is different from the literature, where it is conventionally stated that severe mental deficiency is a hallmark of this syndrome. Moreover, 9/48 (18.7%) patients were able to walk with support (between age 2-12 yrs), whereas 13/48 (27.1%) patients were able to walk unassisted (between 2 1/2-7 yrs). Five/48 (10.4%) patients also achieved diurnal sphincter control. Five/48 (10.4%) patients became self-feeders between age 4-12 yrs. The 34/48 (70.1%) patients receiving serial EEG studies showed fairly distinctive abnormalities, usually outlasting seizures. Although difficult to control in early years, seizures tend to disappear with age. A slow, but constant progress in development was observed in all cases, during the follow-up period. In conclusion, our combined cases represent considerable experience, providing new information on several aspects of this important deletion syndrome.

Pierre Robin sequence and microdeletion 2q32. *C. Houdayer*¹, *M-F. Portnoi*², *V. Soupre*³, *F. Vialard*², *Y. Le Bouc*⁴, *R. Couderc*¹, *M-P. Vazquez*³, *A. Munnich*⁵, *M. Bahuaui*^{1, 3}. 1) Biochimie Biol. Moléculaire, Hôp. Trousseau, Paris, France; 2) Cytogénétique, Hôp. St-Antoine, Paris, France; 3) Chirurgie Maxillofaciale, Hôp. Trousseau, Paris, France; 4) Explorations Fonctionnelles, Hôp. Trousseau, Paris, France; 5) Génétique Médicale, Hôp. Necker Enfants-Malades, Paris, France.

The Pierre Robin sequence (PRS) consists of the nonrandom association of micrognathism, cleft palate (CP) and glossoptosis. Respiratory and feeding difficulties are common and appear to be not merely mechanical but also neurogenic in causation. PRS may be part of a well delineated syndrome, or present as an isolated nonsyndromic entity. PRS is mainly a sporadic finding though rare familial instances may occur, sometimes including individuals with CP only. On the basis of rare chromosome deletions and translocations, a locus for CP could be defined in 2q32. Here, we report a female patient showing the association of nonsyndromic PRS with microdeletion 2q32. Family history was unremarkable and moderate intrauterine growth retardation was noted. In addition to full-blown PRS, the examination showed axial hypotonia and minor physical anomalies such as small low-set ears. An unbalanced reciprocal translocation 46,XX, t(2;21), del 2(q32.3q33.2) was found in the proband's lymphocytes. Her father's karyotype was normal whereas her mother's showed a complex apparently balanced rearrangement 46, XX, t(2;21)(q33.2;q21.2) ins(4;2)(p14;q32.3q33.2). The chromosomes were analyzed by high-resolution G-banding as well as FISH approaches. Hemizygosity analyses using a set of 25 fluorescently-labeled microsatellite markers mapped the deletion between D2S355, telomeric, and D2S2173, centromeric. This observation suggests that some PRS cases might originate from mutation of (a) gene(s) localized in a region hitherto known for harboring a gene for CP. Whether mutations of a single gene mapped to 2q32 could cause both CP and PRS, a possibility suggested by families with mixed heredity, remains to be determined.

Aberrant human genome without phenotypic consequences. *R.S. Verma, M.J. Macera, T. Shklovskaya, S.M. Kleyman.* Division of Molecular Medicine and Genetics. Wyckoff Heights Medical Center, Brooklyn New York Hospital/Weill Medical College of Cornell University, New York, N.Y.

The clinical consequences of an aberrant chromosome have been correlated with as small as a single sub-band. In some cases, duplicated or deleted single bands have resulted in distinct syndromes, while in other situations, the same duplicated and deleted bands have no phenotypic effects. There are a few reports that describe a small inherited duplication of chromosomal regions including 1q21, 1p21-31, 2q11.2-q21.1, 6p23-pter, 6q22-q24, 7p12-p13, 8p23.1, 9p12, 9p22-p24, 9q12, 9q13-21.1, 14q13-q22, 15q11-13, 15q12, 18p11.2-3, and 18q12.1. Similarly, patients have been observed with a variety of deletions for various chromosomal segments including 3p25.3, 3p26, 5p14, 6q23.3-q24.2, 10p14, 11p12, 13q21, 16q21, and Xq26. These findings have created confusion and anxiety in the minds of clinicians and counselors. A 35 year-old normal female was referred because her sister has an unbalanced translocation between chromosomes 4 and 8. Cytogenetic findings with G-banding and FISH-techniques revealed an abnormal karyotype in her peripheral blood: 46,XX, del(3)(p26).ish (TelVysion 3p-). The deleted chromosome was missing the telomere-associated sequences, thus establishing the fact that band p26 was missing on chromosome 3. However, TTAGGG telomere cap was apparently present (Pan telomere probe, Cytocell). In general, ascertainment of such individuals has been through abnormal progenies, and genomic imprinting is the most plausible explanation for abnormal expression. It has been hypothesized that the G-positive bands are rich in repetitive DNA sequences and thus are genetically inert. It is only the G-negative bands which have an apparent adverse effect. Presently, she is expecting with the same chromosome del(3) present in her fetus. These findings have dire consequences for both clinicians and counselors. A concise compendium with detailed clinical manifestations of such cases is being annotated.

A novel mutation in exon 22 underlies a mild cognitive phenotype and atypical renal features in a patient with oculocerebral renal syndrome (OCRL). *A.L. Gropman¹, S.W Levin^{2,3}, L. Yao³, T. Lin⁴, S. Suchy⁴, S. Sabnis⁵, D. Hadley¹, R.L. Nussbaum^{1,4}.* 1) Neurogenetics Branch, NHGRI/NIH, Bethesda, MD; 2) Heritable Disorders Branch, National Institutes of Child Health and Human Development, NIH, Bethesda, M.D; 3) Department of Pediatrics, Walter Reed Army Medical Center, Washington, D.C; 4) Laboratory of Genetic Disease Research, NIH, Bethesda, M.D; 5) Armed Forces Institutes of Pathology, Washington, D.C.

The oculocerebrorenal syndrome of Lowe, (OCRL), is an X-linked disorder characterized by congenital cataracts, mental retardation and renal tubular dysfunction. The gene encodes a lipid phosphatase, phosphatidylinositol 4,5, bisphosphate [PtdIns(4,5)P₂]5-phosphatase, which localizes to the Golgi apparatus and is suspected to play a role in Golgi vesicular transport [Suchy, et al.,1995]. In addition to the ocular and renal manifestations, the majority of boys with OCRL have cognitive problems as well as maladaptive behaviors including tantrums and stereotypies. We report a boy with a history of congenital cataracts and mild developmental delay was also found to have hematuria with proteinuria but minimal signs of renal tubular dysfunction. Subsequent renal biopsy was compatible with a diagnosis of a noncomplement fixating chronic glomerulonephritis. Despite the atypical renal findings, skin fibroblast analysis for PtdIns (4,5P₂ 5-phosphatase was performed and enzyme activity was low, consistent with the diagnosis of OCRL. Mutation analysis using denaturing high performance liquid chromatography (DHPLC) and sequencing revealed an insertion of T between bases 2498 and 2499 in exon 22. The mutation changes a glutamic acid to a stop codon and results in a truncated protein. Western blot analysis from cell lysates showed the ocrl protein was decreased in size and amount. Subsequent screening of the proband's mother revealed her to be a carrier, despite a normal ophthalmic examination. The possibility of OCRL should be considered in boys with cataracts and glomerular disease, even in the absence of renal tubular defects and frank mental retardation usually associated with the syndrome.

Neurofibromatosis in a brazilian reference center : clinical profile and natural history survey. *M. Geller^{1,3}, G. Carakushansky², A. Bonalumi Filho¹, F. Pereira Nunes¹, F. C. Franca¹, D. Rubem Azulay⁴.* 1) Microbiology/Immunology, Teresopolis School of Medicine, Rio de Janeiro, RJ; 2) Department of Pediatrics, UFRJ, Rio de Janeiro, RJ; 3) National Neurofibromatosis Foundation, Inc., USA; 4) Hospital Geral da Santa Casa da Misericórdia do RJ, Rio de Janeiro.

Neurofibromatosis (NF) is a genetic condition with two distinct clinical presentations: NF1 and NF2. The first is one of the most common dominantly-inherited genetic disorders, occurring once in every 3000-4000 people. It is characterized by the occurrence of cafe au lait spots, neurofibromas, plexiform neurofibromas and axillary or inguinal freckling. Other features include an increased risk for optic glioma, osseous lesions, and learning disability. DNA-based testing of the NF1 gene (chromosomal locus 17q11) is available clinically, but is rarely utilized for diagnosis. Prevalence for NF2 is only 1:37,000 individuals. It is characterized by bilateral vestibular schwannomas with associated hearing loss. Other findings include schwannomas of other cranial and peripheral nerves, meningiomas, and juvenile posterior subcapsular cataract. The authors evaluated 46 consecutive children and adults with NF1 and NF2 between June 1998 and April 2000. This prospective study represents the first brazilian clinical survey of patients with NF fully assessed by the the Natural History International Multicentric Protocol under the coordination of the Clinical Genetics Program of Harvard University. The protocol included evaluation of several variables such as age, sex, socio-economic status, disease severity, family history, and the presence of learning disabilities. Complementary exams of affected systems were routinely performed. Our sample was characterized by interfamilial differences in disease severity and tumour susceptibility. Neoplasms alterations which required surgical treatment occurred in 4 patients (8.69% of the sample) and included meningiomas and schwannomas. The diverse array of clinical manifestations found in our sample was analyzed and compared with those described in the literature. A team approach, coordinating the expertise of multiple specialties is strongly recommended for the evaluation of NF patients.

DEB test in mexican patients with atypical manifestations of Fanconi anemia. *C. Esmer, S. Sanchez, L. Gomez, S. Ramos, B. Molina, S. Frias, A. Carnevale.* Department of research in human genetics, National Inst of Pediatrics, Mexico.

Fanconi anemia (FA) is characterized clinically by pancytopenia, hyperpigmentation, skeletal malformations, small stature, diverse congenital abnormalities and predisposition to neoplasia. It has been recognized that the FA phenotype is extremely variable, making diagnosis difficult on the basis of clinical manifestations alone. Hypersensitivity of FA cells to the clastogenic effect of DNA cross-linking agents such as diepoxybutane (DEB) provides an unique marker for the FA genotype and constitutes an useful diagnostic test for FA before anemia develops as well as after the beginning of hematological manifestations. The majority of patients are diagnosed between 3-7 years of age, however the diagnosis of FA is frequently delayed until the pancytopenia appears. In this study we included forty patients with clinical conditions related to the FA phenotype that are not usually screened by the DEB test and we performed the chromosomal breakage test in order to make an earlier diagnosis of the disease. The DEB test was done in a peripheral blood sample by standard procedures in ten patients with aplastic anemia, ten patients with VACTER association, six with radial ray abnormalities, three with tracheoesophageal fistulae, eight with anal atresia and three with myelodysplastic syndrome. Among them we found four DEB-positive patients: Two of them were male patients, twelve and eight years old each, in which their only clinical complaining was aplastic anemia without any other sign of FA. The third patient was a female who has anal atresia, renal hypoplasia, preaxial polydactyly and normal blood cell counts that was diagnosed as VACTER association. The fourth patient was a newborn male showing bilateral radial ray aplasia but lacking other physical or hematological signs. Our results remark the need to amplify the number of abnormalities that indicate when a patient has to be tested for chromosomal instability. Delay in the diagnosis of FA may have serious consequences for the patients and their family.

Iris coloboma with ptosis, hypertelorism, and mental retardation: report of six Brazilian patients. *C.A. Kim, S.M.M. Sugayama, L.M. Albano, C.E. Andrade, T.C. Lyra-Netto, P.S. Gerritsen Plaggert, D.R. Bertola.* Dept Pediatrics, Inst da Crianca - USP, Sao Paulo, Brazil.

In 1988, Baraitser and Winter described a new syndrome with iris coloboma, ptosis, hypertelorism, broad nasal and epicanthal folds in two sibs and an unrelated single patient. Similar phenotype was described in two patients with inversion of chromosome 2 inv(2)(p12q14) by Ayme et al(1979) and Pallota(1991). Ramer et al(1995) assembled 9 children with similar phenotype and two of them presented the same pericentric inversion of chromosome 2. We studied six Brazilian patients (4F;2M) with strikingly similar dysmorphic facial features. They presented hypertelorism(6/6), palpebral ptosis (6/6), broad nasal bridge(6/6), trigonocephaly(3/6) and coloboma(2/5). Other associated findings were cardiac defects(4/6), urogenital anomalies(3/6), umbilical hernia(2/6), and cryptorchidism(2/2). Hip dislocation, camptodactyly, and tapered halux was present in one patient. Mental retardation was present in 4/6(66%). All patients were sporadic cases from non-consanguineous parents. Chromosome analysis were normal except one with pericentric inversion of chromosome 9, also present in his mother. The oldest patient, a 19 year-old girl, had mild to moderate mental retardation and seizures. She maintained the same facial dysmorphisms. There is some phenotypic overlap with patients described by Richieri-Costa et al (1999) and Masuno et al (2000) with frontonasal dysplasia, macroblepharon, eyelid colobomas, ear anomalies, macrostomia, mental retardation and CNS structural anomalies. It is possible that both conditions could represent variable expressivity of the same disorder. Further reports are necessary to clarify the spectrum of this rare condition.

Hirschsprung's disease associated with high birth-weight and coarse facial features - a new syndrome? *I.F.M. Lo, F.W.F. Lam, E.W.L. Hau, H.H.N. Woo, S.T.S. Lam.* Clinical Genetic Service, Department of Health, Hong Kong SAR, China.

Two patients with similar features of Hirschsprung's disease, high birth-weight and coarse facial features were described. The concurrence of these features is never reported before and suggests a new syndrome.

Patient 1 is a 7-year-old Chinese boy born to normal, non-consanguineous parents at 41 weeks of gestation, with birth-weight 4.05 kg. He presented with intestinal obstruction and was diagnosed to have Hirschsprung's disease and Meckel's diverticulum at 2 weeks. Coarse facial features were noticed, together with hypertelorism, epicanthi, protruding tongue, and full cheeks. He was otherwise healthy with normal neurodevelopment. However, his head circumference, height and weight were all along above the 97th percentile. Karyotyping showed normal 46,XY. Bone age at the chronological age of 17 months was appropriate.

Patient 2 is a 21-year-old Chinese gentleman. His parents are non-consanguineous. The mother was mentally slow but otherwise normal. He was born full term with birth-weight 3.9 kg. Hirschsprung's disease was diagnosed at age of 3 months. He was hypotonic with delay in gross motor development in childhood. His intelligence was normal. He suffered from bilateral retinal detachment of unknown cause at age 7. Assessment at age 20 revealed coarse facial features with malar hypoplasia, broad nose, long philtrum, and thick lips, and a stocky built with head circumference 57.5 cm (75th percentile), height 174 cm (50-70th percentile), and weight 98 kg (>97th percentile). Karyotyping showed normal 46,XY.

The differential diagnoses include the well-known overgrowth syndromes like Beckwith-Wiedemann syndrome (BWS), Simpson-Golabi-Behmel syndrome (SGBS), Sotos syndrome (SS), Weaver syndrome (WS), and Perlman syndrome (PS). The one syndrome that bears a similar phenotype to that of our patients is SGBS. However, SGBS patients are, unlike in our patients, usually mentally retarded. Furthermore, Hirschsprung's disease was never reported in SGBS patients previously.

Report of 23 cases of Leber Congenital Amaurosis in Lore Tribe of Sirjan, Kerman, IRAN. *M.H. Karimi-Nejad¹, M.R. Meshkar², S. Sohbati¹, H. Najmabadi¹, R. Karimi-Nejad¹, S. Saryazdi², M. Sarfarazi³.* 1) Pathology & Genetics Ctr, Tehran, Iran; 2) Ophthalmology dept. Kerman Medical Science University. Kerman, Iran; 3) University of Connecticut Health Center, Framington, Connecticut USA.

We have been informed that among the Lore tribes of the rural areas of Sirjan city; a kind of congenital blindness is so frequent, that they are named the blind branch (Tayefehe Couran). At first investigation we concluded that the blindness is hereditary; transmitting as an autosomal recessive trait.

We did several field studies, examined the affected individuals, interviewed the Seniors, drew pedigree charts going back 11 generations. We ascertained 23 patients, 8 of whom were dead. We did G Banded chromosomal study, took blood samples of the patients and their first degree relatives, including parents, and sibs, for DNA analysis and Linkage analysis. The materials are under study.

The 12 living patients ranging from 6-63y, mean age 21.5, were fully worked up by our clinician and ophthalmologist. The blindness is recognized in the early neonatal period, without noticeable objective findings (12/12).

Nystagmus (8/12), Keratoconus: 3 bilateral, 2 unilateral; leukoma: 2 bilateral, 1 right; and 2 bilateral and one right eye cataract appearing later on.

On fundus visualization narrowing of retinal vessels (9/12), dispersed pigment deposition (3/12), white spot, and retinal degeneration (1/12) were remarkable. Electro retinogram of 2 cases revealed characteristic pattern.

Blindness is profound, ranging from 2/10 one case, finger count 2/12, hand movement 3/12, light perception 5/12, to complete blindness with NLP in one. Eye tension were normal in cases that could be examined.(4/12)

We think that we are dealing with Lebers Congenital Amaurosis. We are still working on identification of the genes molecular structure and its chromosomal mapping.

This research is being sponsored by the National Iranian Research Organization.

Violating the law of parsimony: independent segregation of facioscapulohumeral dystrophy, autosomal recessive deafness, and mental retardation in a single sibship. *L.E. Walsh^{1,2}, E.C. Lichtenberg¹, B.P. Garg²*. 1) Dept Medical & Molecular Gen, Indiana Univ Sch Medicine, Indianapolis, IN; 2) Dept Neurology, Section of Pediatric Neurology, Indiana Univ Sch Medicine.

Facioscapulohumeral dystrophy (FSHD) is an autosomal dominant disorder consisting of muscle wasting and progressive weakness about the face, neck and shoulder girdle; progressive sensorineural hearing loss is seen in 60-70% of patients and cognitive disability has been reported. We report a nuclear family, including 10 siblings, with weakness, deafness, and mental retardation. The mother had progressive shoulder weakness but no hearing loss or history of learning disorders. Her 10 children ranged in age from 7-20 years. Within this sibship, 2 (1 boy, 1 girl) had only shoulder girdle weakness; 2 others (1 boy, 1 girl) were deaf but otherwise normal. Two boys were mentally retarded with onset of hypotonia in infancy; 1 was deaf, but had required extracorporeal membrane oxygenation as a newborn. One girl was mildly learning-disabled, had normal hearing, and questionable shoulder weakness at age 16, while a 13-year-old boy had only weakness by report, but has not yet been examined. One boy had only mild learning disabilities; 1 girl was normal. Family history was otherwise negative for neuromuscular disorders, hearing loss, or mental retardation; consanguinity is denied. The mother's RFLP analysis revealed heterozygosity for chromosome 4q35 deletion, confirming a clinical diagnosis of FSHD. Four siblings, the 2 with isolated deafness and the 2 with mental retardation (1 with deafness), tested negative for the deletion. Testing of the hearing-impaired children for other causes of deafness is pending. This family presented with a rare combination of FSHD and probable autosomal recessive deafness, which are both relatively uncommon. The coexistence of these two conditions in this family emphasizes the utility of subtle phenotypic differences as aids to diagnosis. It also raises genetic counseling issues in the face of possible phenocopies, and emphasizes the medical and psychosocial need for preparatory genetic counseling coupled with definitive genetic testing.

Orofacial Clefts and Associated Congenital Malformations. *S. Beiraghi¹, C.M. Chan¹, S.A. Jensen¹, G.B. Schaefer².*
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The types of associated malformations with orofacial clefts vary widely between studies. The purpose of this investigation was to evaluate the incidence of associated congenital malformations of orofacial cleft patients in a geographically defined population.

Medical records of 1127 orofacial cleft patients at the cleft palate/craniofacial clinic at Boys Town National Research Hospital were reviewed between the years 1990-1999. All patients had a genetic evaluation and a complete laboratory workup. Patients were divided into two categories: 1) cleft palate only, and 2) cleft lip, with or without cleft palate. Patients were further categorized in respect to occurrence and type of congenital malformations occurring in conjunction with the orofacial clefts.

Of the 1127 cleft patients, 47.2% were born with cleft palate only and 52.8% with cleft lip, with or without cleft palate. Thirty two percent of all cleft patients had associated congenital malformations. Associated malformations were more common in patients who had cleft palate only (38.7%) than in patients with cleft lip, with or without cleft palate (26.4%). Malformations in the facial region were the most common followed by malformations in the cardiovascular, central nervous, and skeletal systems. Of the major malformations 63.1% were diagnosed as occurring in conjunction with chromosomal or syndromic etiologies, while 36.9% did not. These results correlate closely with results from other recent orofacial cleft studies.

The recognition that there is a high association of organ malformations and syndromes with orofacial clefts is vitally important for clinicians. The identification of orofacial clefts should lead to a complete genetic examination of an individual and, if indicated, further diagnostic testing and counseling.

Midline cervical cleft, a rare malformation of branchial arch derivation. *D.L. Earl, R.E. Falk.* Medical Gen/Birth Defects Ctr, Cedars-Sinai Medical Ctr, Los Angeles, CA.

Congenital midline cervical cleft with associated anterior cervical webbing is a rare developmental defect. This can be associated with other anomalies including median cleft of the chin, lower jaw, lower lip, tongue, and sternum, congenital heart defects and hemangiomata. It results from failure of branchial arch fusion and occurs sporadically. We describe an infant with a congenital midline cervical cleft and mild cervical webbing born to a healthy G3P2-3, 31-year-old woman and her healthy, unrelated 31-year-old partner. Due to an elevated MS-AFP of 2.53 MoM, ultrasound-directed amniocentesis was performed at 17 weeks gestation. No fetal abnormalities were noted. Cytogenetic analysis revealed a normal male karyotype. The AF-AFP result was normal (1.55 MoM). The infant was delivered at term. Birth weight 3550 gm, length 48.25 cm, and head circumference 35.5 cm were 50th-90th percentiles. He was non-dysmorphic with slightly overfolded superior helices, prominent antihelices, mild retrognathia, and a palpable midline notch of the mandible. A midline vertical soft tissue lesion extended from the hyoid process to above the sternal notch, measuring less than 2.0 cm with the head in a neutral position. With the neck extended, a V-shaped pterygium was visualized reaching from the cleft to the mandible. No discharge, other midline defect, abdominal raphe, cardiac murmur or hemangioma was noted. There were no problems of neonatal adaptation and the infant has continued to grow and develop normally. Newborn hearing screen and thyroid function studies were normal and there was no evidence of a thyroglossal duct cyst or remnant on ultrasound of the neck. Fewer than 50 cases of midline cervical cleft have been reported including 12 cases from a 30 year survey of 612 thyroglossal duct and branchial cleft sinus surgical referrals (Gargan TJ, et al, 1985). Cicatricial scarring leads to progressive flexion contracture of the head and secondary mandibular constraint in untreated cases. Thus, it is important to recognize this rare developmental defect and refer for appropriate early surgical management. Detailed clinical findings and literature review will be presented.

Mapping the gene for Raine syndrome, a rare lethal autosomal recessive condition. *A. Ion¹, C. Tipper¹, A. Crosby¹, K. Marks¹, R. Taylor¹, I. Jeffrey², H.M. Kingston³, J.M. Pina-Neto⁴, R. Sachdev⁵, M.A. Patton¹, S. Jeffery¹, V. Murday¹.* 1) Medical Genetics, St George's Hospital Medical School, London, UK; 2) Histopathology, St George's Hospital Medical School, London, UK; 3) Clinical Genetics, St Mary's Hospital, Manchester, UK; 4) Genetics, Faculdade de Medicina de Ribeirao Preto, Sao Paulo, Brasil; 5) Clinical Genetics, Churchill Hospital, Oxford, UK.

Raine syndrome is a genetic condition characterised by multiple congenital abnormalities including bone dysplasia, microcephaly, characteristic dysmorphic face, hypoplastic lungs, and in some cases, hepatosplenomegaly. Affected children usually die soon after birth from respiratory distress or cardiac arrest. An autosomal recessive mode of inheritance has been suggested for this disorder since consanguinity in the parents has been demonstrated in several of the reported cases. Since 1989 (when first described by Raine et al.), only 9 such patients have been reported, and the aetiology and physiopathology of this condition are completely unknown. We describe a new case of Raine syndrome, from unrelated parents, presenting with a rearrangement of the short arm of the chromosome 7. The karyotype abnormality resulted in uniparental isodisomy and the loss of a very small fragment from both chromosome 7 homologues. The loss was confirmed by both molecular and FISH techniques, and a YAC/cosmid contig was generated for the deleted fragment as well as for the proximal non-deleted region. At present, only two ESTs have been mapped to this area. To further refine this putative locus, homozygosity mapping was performed in three other cases of Raine syndrome (one reported by Kingston et al. in 1991 and two previously undescribed patients). Candidate genes in this region are discussed.

An infant of agnathia, situs inversus, and no brain malformation. *T. Tohma^{1,2}, Y. Asato^{1,2}, Y. Chinen¹, Y. Izumikawa³, K. Sakumoto², T. Ohta¹, K. Naritomi⁴.* 1) Dept Pediatrics, University of the Ryukyus School of Medicine, Okinawa, Japan; 2) Maternity and perinatal care center, University of the Ryukyus hospital, Okinawa, Japan; 3) Dept Pediatrics, Nago Institute for Severely Handicapped Children, Okinawa, Japan; 4) Dept Medical Genetics, University of the Ryukyus School of Medicine, Okinawa, Japan.

Agnathia is a very rare congenital anomaly considered to be a lethal condition. We report here a 9-month-old Japanese boy, who is the third survival case. At birth, he was noted to have extreme micrognathia, microstomia, low-set ears, and downward-slanting palpebral fissures. There were no spontaneous respirations. After several trials, intubation with bronchofiberscope was successful after 10 minutes. Apgar scores were 3 at 1 minute and 6 at 5 minutes. He had situs inversus and no brain malformation. A tracheostomy and a gastrostomy were performed at 2 weeks and 2 months respectively. At 9 months of age, his mental development appears almost normal. An infant of agnathia and situs inversus who had no significant brain malformation had been reported. But she died at 5 days of age because of severe hyaline membrane disease (Stoler et. al., 1992). To our knowledge, there have been reported only 2 survival cases, who were 5-year-boy and 14-month-old girl (Kamiji et. al., 1991, Walker et. al., 1995, respectively). Both of them had neither brain malformation nor situs inversus. Our patient differs from these cases in that he had situs inversus.

Congenital anomalies: a review of 279 consecutive autopsies in Ribeirao Preto, Brazil. A. Píram¹, L.C. Peres², J.M. Pina-Neto¹. 1) Dept Genetics, School Medicine of Ribeirao Preto, Sao Paulo University, Ribeirao Preto, SP, Brazil; 2) Dept Pathology, School Medicine of Ribeirao Preto, Sao Paulo University, Ribeirao Preto, SP, Brazil.

We retrospectively analyzed 1127 autopsy of children aged 0 to 14 years, performed in the Pathology Service of the University Hospital of School of Medicine of Ribeirao Preto from January 1995 to December 1998. Congenital anomalies were present in 279 cases (24.8%). Most cases were children aged 0 to 28 days (40.9%), and 1 to 12 months (36.2%). Sample was separate in two groups: evaluated for clinical geneticist in life or autopsy (157 cases) and not evaluated (122 cases). In the group of evaluated cases the diagnosis can be established in 126 (80.3%); 21 (13.4%) were of single anomalies, and 105 (66.8%) of multiple anomalies. In this group the diagnosis was not defined in 31 cases (19.7%), being 13 (8.3%) of supposed single anomalies and 18 (11.5%) of multiple anomalies. In the group of not evaluated cases 62 (50.8%) had the diagnosis defined; 28 (23.0%) were single anomalies, and 34 (27.9%) were multiple ones. Diagnosis cannot be defined in 60 cases (49.2%); 38 (31.1%) were of supposed single anomalies and 22 (18.0%) of multiple anomalies. Considering the cases with established clinical diagnosis (188 cases), 40 (21.3%) were monogenic, 33 (17.6%) chromosomal, 38 (20.2%) multifactorial, 6 (3.2%) environmental, 54 (28.7%) unknown etiology, and in 17 (9.0%) the etiology cannot be determined. The recurrence risk was considered low in 121 cases (64.4%), moderate in 21 (11.2%), high in 28 (14.9%), dependent of handling in 1 (0.5%), unknown in 7 (3.7%) and not defined in 10 (5.3%). The cases without certainty diagnosis (91 cases), had the etiology and the risk considered as undetermined. Failure in reaching goals in most cases, resulted from the not accomplishment a full protocol that includes evaluation for clinical geneticist, photographic and radiological documentation, cytogenetic and molecular studies. The authors demonstrate that collaborative work between Genetics and Pathology Services can succeed for clinical diagnosis, etiology and recurrence risks definition. Financial Support: FAPESP.

Probable autosomal dominant aqueductal stenosis. *S. Sastry*¹, *E. Schneider*², *J. Halperin*³, *M.G. Bialer*¹. 1) Dept. of Pediatrics; 2) Ob/Gyn; 3) Neurology; North Shore Univ. Hospital/NYU School of Medicine, Manhasset, NY.

A 30 yr old Hispanic female presented at 19 wk gestation with sonogram findings of moderate to severely dilated lateral ventricles and mildly dilated 3rd ventricle with no other abnormalities, consistent with a diagnosis of aqueductal stenosis (AS). Amniotic fluid AFP was normal and amniocyte chromosome analysis was 46,XX. The mother's medical history was significant for seizures, but she was not on medications as her last seizure was at 18 yr. A CT scan done at 18 yr showed mild hydrocephalus. She also had an anal anomaly repaired at birth. On physical exam she had a left single transverse palmar crease with thenar hypoplasia, cupped ears and a small right ear. She had no cognitive or physical limitations. An MRI scan performed on her during the pregnancy showed significant obstructive hydrocephalus involving the lateral and 3rd ventricles, compatible with a diagnosis of AS. The corpus callosum was thinned but no other abnormalities were detected. The baby was delivered by planned C-section at 34 wk gestation due to oligohydramnios. Birthweight was 1905g (25%), length 41 cm (<3%) and OFC 32.7 cm (75-90%). Anterior and posterior fontanelles were large with prominence of the forehead. No other abnormalities were detected. MRI scan confirmed AS. A VP shunt was placed. Results of L1CAM testing are pending. At 1 year, the baby is babbling with specific "ma-ma" and "da-da", is able to stand with assistance and cruises in a walker. She is somewhat small for age but growing proportionately. This history is consistent with autosomal dominant AS. Hydrocephalus is seen in approximately 1/1000 pregnancies, of which about 40% are AS. There is an X-linked recessive form caused by mutations in the L1CAM gene and other cases may be multifactorial. We are aware of 2 families with apparently autosomal dominant AS with 5 affected individuals (J Neurolog Sci 1998;158:101). 4/5 individuals are intellectually normal. The 5th is a 2.5 yr old child with developmental delays. Although AS prenatally diagnosed at this gestational age has a poor prognosis, the outcome in this case has been encouraging, and autosomal dominant AS may have a better prognosis than other forms.

Otopalatodigital TypeII in a female: clinical and laboratory studies. *S. Guarducci, A. Cecconi, E. Lapi, G. Ricotti, U. Ricci, M. Lapini, L. Ombroni, E. Andreucci, S. Pelagatti, V. Lazzerini, I. Sani, A. Brusafferri, M.L. Giovannucci Uzielli.* University of Florence, Department of Paediatrics, Azienda Meyer, Genetics and Molecular Medicine Unit, Florence, Italy.

Otopalatodigital Syndrome (OPD), subdivided into type I (MIM 311300) and type II (MIM 304120) is a rare genetic disorder, transmitted as an X-linked recessive trait. OPD II is a potentially lethal skeletal dysplasia, characterized by abnormal features at the facial and extremities level, and by multiple skeletal abnormalities. Thoracic hypoplasia may lead to early death (Nishimura et al. 1997). The phenotypic overlap among OPD type II and Melnick-Needles syndrome, a disorder transmitted as an X-linked dominant trait, and also Larsen syndrome, may justify the expansion of these different entities. We recently encountered a female newborn, with specific and severe clinical manifestations of OPD type II. Cytogenetic and cytogenetic-molecular analyses revealed a normal 46, XX karyotype, whereas molecular study excluded UPD, and X-inactivation analysis failed to demonstrate a skewed inactivation.

Evaluation of clinical and molecular criteria in 240 patients affected with Hereditary Motor and Sensory Neuropathy (HMSN). *R.B. Bernard¹, A. Navarro¹, J. Pouget², C. Desnuelle³, N. Philip^{1,4}, N. Levy^{1,4}.* 1) Departement de Genetique Medicale, Hopital de la Timone, Marseille; 2) Service des maladies neuromusculaires, Hopital de la Timone, Marseille; 3) Service de reeducation fonctionnelle, Hopital l'Archet, Nice; 4) Inserm U491, Marseille, FRANCE.

We studied 240 patients affected with neurologic features consistent with HMSN. Our study was conducted on the basis of clinical, genealogical, electrophysiological, histologic and molecular criteria. By using several molecular technical approaches and particularly a new CMT1A-REPs PCR approach, CMT1A duplications were identified in 25% of patients. We evidenced that the association of 5 major phenotypic criteria increases the probability to detect the 17p11.2 duplication (increased risk X 8). Interestingly, we demonstrate the age of diagnosis in CMT1A is significantly higher in females than males, suggesting the existence of sex-depending factors influencing the disease severity. Eventually, we evidenced a positive correlation between age of onset in CMT1A and motor VCN values. Our data confirm the clinical heterogeneity of the disease, essentially within the same family. A 17p11.2 deletion responsible for HNPP was detected in 14% of patients. None of the HNPP clinical criteria was specific for positive diagnosis. Oppositely, increased distal latencies evaluated by electrophysiology are more contributive. Presence of non-neurological features associated to pressure palsies drastically reduces the probability of HNPP deletion. Point mutations in Connexin 32 gene were identified in 12 patients with common clinical phenotype of CMT1. In such cases, electrophysiological findings are usually atypical. A search of point mutations in P0, PMP22 and EGR2 was conducted in patients affected with severe phenotypes. Very rare mutations in these genes were identified. Our results suggest that prospective studies of the genotype-phenotype correlations in CMT1A disease are still necessary to evidence predictive criteria of severity. This approach will allow to identify families in whom modifier genes could segregate. They also evidence that molecular strategies to explore HMSN have to be supported by precise phenotypic informations.

Clinical and Molecular Studies in a large unique family with Limb-Girdle Muscular Dystrophy and Paget

Disease of Bone. *V.E. Kimonis¹, M.J. Kovach¹, S. Leal⁴, B. Waggoner¹, R. Khardori², D. Gelber³.* 1) Dept Ped, Div Gen & Metabolism, Southern Illinois Univ Sch Med, Springfield, IL; 2) Dept of Internal Medicine, Southern Illinois Univ Sch Med, Springfield, IL; 3) Dept of Neurology, Southern Illinois Univ Sch Med, Springfield, IL; 4) Lab of Statistical Genetics, Rockefeller University, NY, NY.

A large Central Illinois family with Limb-Girdle Muscular Dystrophy (LGMD) and Paget disease of bone (PDB) includes 11 affected individuals (8 M, 3 F) ranging in ages from 33 y to 64 y. Onset of PDB is at a mean age of 41 y with pain in the hips, shoulders and back. Onset of the LGMD is at a mean age of 45.6 y. with weakness of the girdle muscles, and absent/reduced tendon reflexes. Affected individuals have elevated alkaline phosphatase (mean 455 (normal 30-130 mg/dl)), and CPK levels (mean 273 (20-222 mg/dl)). High-resolution karyotype is normal. Muscle biopsies in 5 individuals reveals non-specific myopathy with vacuolar changes seen in the oldest male. Individuals die prematurely from progressive muscle weakness, cardiomyopathy and cardiac failure in the forties to sixties. There are three previous reports of PDB and neuromuscular disease. Caughey et al. (1957) described familial dominant dystrophia myotonica, Tucker et al. (1982) identified a family with dominant amyotrophic lateral sclerosis, and McBride et al. (1966) found recessive LGMD in four of six siblings in addition to PDB. Linkage analysis in this family excluded all the 22 candidate loci for LGMDs, myopathy, PDB and cardiomyopathy. A genome wide search (Marshfield) indicated linkage to a unique locus on 9p21-q21 with D9S301 (max LOD=3.64) which includes the locus for recessive vacuolar myopathy, IBM2, however excludes the LGMD2H locus. Identification of the specific gene mutation may permit understanding the pathogenesis and permit development of specific treatment protocols in this unusual disorder.

High frequency of Klinefelter syndrome among a large population of patients referred for Fragile X syndrome testing due to undiagnosed mental retardation. *M. Khalifa, J. Struthers.* Dept Pediatrics and Pathology, Queen's University and Kingston General Hospital, Kingston, Ontario, Canada.

Approximately 3% of the general population have mental retardation, with the majority of cases in the mild range. About 40% of cases with mental retardation remain without a specific diagnosis. In this study, we screened 1619 DNA samples from 1217 patients referred for DNA testing for Fragile X syndrome (FRAX), primarily because of mental retardation, hyperactivity, behaviour problems or learning disability. Our molecular approach for X chromosome aneuploidy screening involved dosage comparison following Southern blotting and cohybridization with two non-polymorphic markers, one on the X chromosome and the other on an autosome. Dosage evaluation was carried out both visually and using densitometric measurement. In normal females (46, XX), the intensity of the two bands should be equal, representing two copies of the alleles on both the X and the autosome. In normal males (46, XY), the X chromosome allele will be half the intensity of the autosomal one. Samples deviating from the expected pattern were retested and confirmatory testing was done. Seven pre-pubertal patients who were referred because of FRAX features or MR were found to have Klinefelter syndrome (47, XXY). Among the same age group, a similar number of patients were found to have FRAX. Since approximately 3/4 of the studied population were males, and 3 of the identified FRAX cases were suspected because of a family history of the disease, Klinefelter syndrome could be the most common cause of undiagnosed mental retardation among pre-pubertal males without a family history of mental retardation or FRAX (~2% versus 1.3% for FRAX). Because of the significant medical and psychological benefits of early recognition of Klinefelter syndrome, we recommend cytogenetic study for all cases of mild mental retardation among pre-pubertal males even without dysmorphic features.

Bartsocas-Papas Syndrome (MIM 263650) in a West African family: Prenatal diagnosis and further delineation.

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Bartsocas and Papas (1972) reported 4 sibs in a consanguineous Greek family with a recessive form of the popliteal pterygium syndrome (MIM 119500). The clinical findings include LBW, ankyloblepharon, cleft lip/palate, filiform bands between the jaws, popliteal pterygium, syndactyly, and nonectodermal anomalies including microcephaly and genital abnormalities. Early lethality is a common feature. We report a case of a Gambian infant with this disorder.

Case Report: SB was the 2165 gm product of a 36 week gestation complicated by IUGR. An ultrasound at 30 weeks revealed a talipes deformity and a large gap between the 1st and 2nd toes. He was delivered by section because of breech presentation to a 19 year old primigravida from Gambia who is the first cousin of the father. The newborn examination revealed a right-sided cleft lip/palate, symblepharon, and synechia between the upper and lower alveoli. He had bilateral cryptorchidism and a large popliteal pterygium. There was syndactyly of digits 2,3,4 of both hands, hypoplasia of the fingernails of the same digits and a large gap between the 1st and 2nd toes secondary to the pterygium. The ankylosed mandible resulted in a tracheostomy and gastrostomy within the first 2 weeks of life. The oral synechia and ocular filiform adhesions were also lysed. A 3-D CAT scan demonstrated probable fusion between the left coronoid and zygoma as well as the condylar head to the temporomandibular fossa. A high resolution karyotype was normal.

All but one of the reported cases (10 males and 8 females) have been from the Mediterranean region. Syngnathia is a rare anomaly involving soft tissue or bony adhesions between the maxilla and the mandible. Only about 60 cases have been reported mostly in association with the popliteal pterygium syndrome and involving soft tissue only. Our patient is the first from Africa, the only case with bony syngnathia, and the first case indicating the practicality of prenatal diagnosis of this disorder.

Unusual findings in a case of Marshall-Smith syndrome. Further delineation of the phenotype. *E. Tron¹, M. Velinov^{1,3}, S. Barrett¹, E. Moran², R. Wallerstein², G. Kupchik¹.* 1) Pediatrics, Maimonides Medical Center, Brooklyn, NY; 2) Clinical Genetics, Hospital for Joint Disease, Orthopedic Institute, NY,NY; 3) NYS Institute for Basic Research, Staten Island, NY.

Marshall-Smith syndrome is a rare disorder of accelerated skeletal maturation, failure to thrive and specific dysmorphic features. Respiratory system complications often cause death in early childhood. We report a female patient who was admitted at 3 years and 9 months of age because of high fever and suspected infection. The physical exam showed dolichocephaly with prominent forehead, blue sclerae, anteverted nares, dysplastic teeth, stridor, pectus excavatum with flared ribs, and generalized hypotonia. Since birth, the patient had extensive diagnostic work up that showed consistently advanced bone age, height at or above 95%, weight at or below 50%, laryngomalacia and duplicated left kidney. Chromosomes were normal: 46,XX. Her metabolic work up and head MRI were unremarkable. The X-ray of hands showed advanced bone age and broad proximal phalanges as well as disharmonious maturation between the carpal bones and the distal aspect of hand. This patient also had history of unexplained high fever causing multiple hospital admissions. Her usual baseline temperature was reported to be 102 degrees. Multiple clinical and laboratory evaluations did not reveal any specific infectious cause for the elevated temperature. There was no evidence of serious chronic or acute respiratory complications during these evaluations. The reported patient has the typical dysmorphic characteristics of Marshall-Smith syndrome. The diagnosis is further supported by advanced bone age, low weight for height and absence of laboratory findings indicating diagnostic alternatives. The unusual finding of consistently elevated body temperature without indication of ongoing infection, suggesting a central regulation abnormality, has not been reported in association with Marshall-Smith syndrome to our knowledge. In addition, our patient did not have serious respiratory complications that are typical presentation of the syndrome. These unusual presentations may help better delineate the clinical features associated with the condition.

Ovarian failure in neutral lipid storage disease (NLSD): report of a case. *D.J. Aughton.* Wm. Beaumont Hospital, Royal Oak, MI.

A 24-year-old woman of Mediterranean descent presented for evaluation of idiopathic hepatomegaly, ichthyosis, sensorineural hearing loss, and primary ovarian failure. There was no known consanguinity. She had a slender habitus but with plethora of the face, neck, and upper thorax. Her skin was dry and fissured.

I did not recognize this constellation of findings. Chromosome analysis yielded normal results. I learned in reviewing past records that when the woman was 4 years old, a dermatologist had rendered a diagnosis of nonbullous congenital ichthyosiform erythroderma; skin biopsy was interpreted as being consistent with lamellar ichthyosis; liver biopsy had shown fatty metamorphosis and minimal fibrosis; and peripheral blood smear had shown vacuolation of granulocytes even though multiple later automated blood counts had been normal. In further reviewing the literature relevant to the ichthyoses, I recognized that the woman's findings were consistent with NLSD. I ordered hematopathologic review of the peripheral blood smear. This showed neutrophils, monocytes, and basophils with multiple small- to medium-size round cytoplasmic vacuoles. I confirmed a diagnosis of NLSD.

NLSD (McK 275630) is a rare autosomal recessive condition that is characterized by the presence of nonmembrane-bound cytosolic droplets in a wide variety of cells. Manifestations may include ichthyosis, hepatosplenomegaly, and sensorineural hearing loss as well as neuromuscular, ocular, and other abnormalities. Vacuolated neutrophils are present in all patients; however, automated analyzers often do not identify these, and this valuable clue is thus easily overlooked. I know of no previous reports of joint occurrence of ovarian failure and NLSD; however, only 7 of the 26 cases described in a recent review (*J Pediatr Gastroenterol Nutr* 1997;25:541-547) were women of reproductive age. I do not know with certainty that the woman's ovarian failure is a direct consequence of her NLSD, but the multisystem nature of and the ubiquitous occurrence of cytoplasmic vacuolization in NLSD strongly suggest a causal relationship.

Homozygosity mapping of a locus for a novel form of syndromic ichthyosis to chromosome 3q27-q28. *L. Baala*^{1,2}, *S. Hadj-Rabia*^{1,3}, *D. Hamel-Teillac*³, *S.M. Leal*⁴, *A. Sefiani*², *Y. de Prost*³, *A. Munnich*¹, *S. Lyonnet*¹, *P. Vabres*^{1,3}. 1) Genetique INSERM U-393, Hopital Necker, Paris, France; 2) Department of Genetics and Molecular Biology, INH Rabat, Morocco; 3) Service de Dermatologie, Hopital Necker, Paris, France; 4) Laboratory of Statistical Genetics, The Rockefeller University, New-York, USA.

Autosomal recessive congenital ichthyoses (ARCI) are a heterogeneous group of disorders characterized by abnormal scaling of the skin. Here, we describe a novel form of syndromic ARCI associated with sclerosing cholangitis, hypotrichosis of the frontal scalp and vacuolated leukocytes and keratinocytes in two boys and a girl born to second cousin parents. Normal biliary ducts on histological examination excluded ichthyosis congenita with biliary atresia. In addition, despite similarities with Dorfman-Chanarin syndrome (DCS), this diagnosis could be also excluded on the basis of: i) absence of muscular or ocular features, ii) hepatic involvement with sclerosing cholangitis as opposed to the fatty infiltration observed in DCS and iii) the presence of small-sized leukocyte and keratinocyte vacuoles that were absent in unaffected relatives. Thus, while this disorder appears clearly distinct from DCS, clinical overlap may reflect a common genetic origin or metabolic pathway. Finally, Refsum disease, another syndrome with ichthyosis and vacuolated keratinocytes, could be excluded as well. A genome wide search for homozygosity revealed that only marker AFM308yf1 at locus D3S1601 was found homozygous in all affected individuals and heterozygous in parents and unaffected sibs. Two-point linkage analyses at this locus gave a Zmax value of 1.93 at q = 0% while a maximum multipoint lod score of 3.0 was obtained across the region of homozygosity loci : D3S3596, D3S1580, D3S2747, D3S1601 and D3S3669. It should be noted that the maximum lod score expected for this pedigree was 3.25. Our data thus strongly suggest that the disease locus maps to a 16.3 cM region on chromosome 3q27-q28, between loci D3S3628 and D3S1265, where no obvious candidate gene has been localized.

Brauer nevus (congenital temporal triangular alopecia) with distichiasis and adjacent facial hyperpigmentation in an African American male. *K.A. Fjelstad, S.J. Hassed, J.J. Mulvihill.* Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Congenital temporal triangular alopecia (CTTA) may be defined as a persistent focal area of hair loss occurring in the temporal or frontal area. A ten-year-old African American male had focal scalp hair hypoplasia and facial hyperpigmentation since an age of three years, but not before. Prior treatment with antifungals and topical corticosteroids had not altered the scalp hair growth. On examination, the patient had two areas of hair hypoplasia in the left frontotemporal region. The anterior edge of one area was continuous with a large whorled, hyperpigmented facial lesion. Hyperpigmented skin was also present in the right popliteal fossa. The patient had bilateral distichiasis (two distinct rows of eyelashes) with long eyelashes in each row. Family history was negative for hair hypoplasia, but there was a report of hyperpigmentation in a second degree relative. Patients with Brauer nevus typically present in early childhood and have been reported in Caucasian and Asian subjects but not in African Americans. CTTA may be subtle and unrecognized and is distinct from alopecia areata, tinea capitis, traction alopecia, and scarring of the scalp. CTTA may be associated with other changes in hair growth and skin pigmentation. A family history of alopecia areata has been reported in five cases of CTTA, but CTTA does not appear to be immune-mediated. Similar skin and eyelash changes are seen in subjects with CTTA and alopecia areata. Eyelash trichomegaly is seen with alopecia areata in subjects with HIV infection; alopecia areata has also been associated with depigmentation or vitiligo. It is tempting to consider Brauer nevus (CTTA) with distichiasis and hyperpigmentation as a pattern of somatic cell mosaicism, but the distal hyperpigmentation argues against that notion. Further identification and study of persons with CTTA may lead to better understanding of the development of disorders of the hair and skin.

Extensive, Acquired Hypopigmentation in a Patient with 22q11 Deletion Syndrome. *J.L. Hand^{1,2}, S.D. Calobrisi³, N.M. Lindor¹*. 1) Dept Genetics, Mayo Clinic, Rochester, MN; 2) Department of Dermatology, Mayo Clinic, Rochester, MN; 3) Boca Raton, FL.

22q11 Deletion Syndrome (also known as Shprintzen or Velocardiofacial Syndrome) is a common chromosomal deletion syndrome that may result in a wide range of clinical findings. We describe a seven-year-old girl who presented with generalized, acquired hypopigmentation and premature graying of the hair. Past medical history was significant for hyperopia since age five, elevated thyroid antibodies, submucous cleft palate and recurrent otitis media requiring myringotomy tube placement. Physical exam revealed hypernasal speech, macrocephaly, coarse thick hair and a bifid uvula. Skin exam showed extensive discrete and confluent macular hypopigmentation over approximately 70% of the body surface area. Hair exam showed graying of single hairs throughout the vertex scalp and eyelashes. Skin biopsy showed hypopigmentation with absence of melanin in the epidermis. Consensus evaluation by several dermatologists was that the findings were atypical for vitiligo. Significant family history included Tetralogy of Fallot and premature graying of the hair in the patient's father and truncus arteriosus in a sister. On laboratory evaluation, karyotype was normal, 46, XX, but a hemizygous 22q11.2 deletion was detected by FISH probe. Echocardiogram, calcium, and phosphorus levels were normal. To our knowledge, this is the first reported case of acquired hypopigmentation associated with familial 22q11.2 deletion. Further cases are needed to determine if premature graying of the hair and hypopigmentation of the skin represents an association with 22q11 deletion or a coincidental finding.

The development of cutaneous neurofibromas is influenced by familial and local factors in patients with Neurofibromatosis 1 (NF1). *C. Palmer¹, J. Szudek¹, H. Joe², V.M. Riccardi³, J.M. Friedman¹*. 1) Dept Medical Genetics and; 2) Dept Statistics, University of British Columbia, Vancouver, Canada; 3) American Medical Consumers, Los Angeles, CA.

NF1 is an autosomal dominant condition affecting 1 in 3000 individuals. Its defining feature is the neurofibroma: a complex benign tumor arising from peripheral nerve sheaths. The number of cutaneous neurofibromas in NF1 patients increases with age and is highly variable; the cause of this variability is unknown. We tested the hypothesis that development of these lesions may be influenced by local and familial factors.

The presence or absence of 1 or more cafe au lait spots, 1 or more cutaneous neurofibromas, and 1 or more diffuse plexiform neurofibromas was recorded for each of ten divisions of the body surface in 768 NF1 patients, including 117 affected individuals in 52 families. We used a random effects model to obtain the maximum likelihood estimate and confidence interval of intrafamilial correlations in the number of body divisions affected with 1 or more cutaneous neurofibromas, while controlling for age. The correlation amongst first-degree relatives was $r=0.30$ (95% CI=0.078,0.52), in agreement with previous studies.

We used a Mantel-Haenszel test, stratified simultaneously by body division and number of body divisions with 1 or more cutaneous neurofibromas, to examine associations in the presence of diffuse plexiform neurofibromas and cutaneous neurofibromas in individual NF1 patients ($n=630$). Divisions that include a diffuse plexiform neurofibroma are twice as likely to have 1 or more cutaneous neurofibromas as well (summary odds ratio=2.02; 95% CI=1.28, 2.77). Odds ratios were not homogeneous across body divisions. No significant association was observed between the presence of cafe au lait spots and cutaneous neurofibromas in a body division in NF1 patients ($n=584$).

We conclude that the occurrence of cutaneous neurofibromas in NF1 patients is influenced by familial factors as well as by local factors.

Reactive perforating collagenosis: a study of a five generation Indian family with autosomal dominant inheritance. *V. Patel¹, T. Mehta², J.V Solanki³, U. Radhakrishna²*. 1) RIA Laboratory, Green Cross Voluntary blood bank and pathology laboratory, Ahmedabad, Gujarat, India; 2) "Samarpan", Centre for skin, sexually transmitted diseases and Research Centre, Sarvoday School, Modasa-383315, India; 3) Department of Animal genetics & Breeding, Veterinary college, Gujarat Agriculture University, Anand, India.

Reactive perforating collagenosis (RPC) is a rare skin disorder characterized by umbilicated papules on the sun-exposed areas of the face, arms, and legs and usually begins in early childhood. We report an Indian family living in the state of Gujarat (India) in which RPC was observed in five generations. The pedigree consists of a total of 139 persons with 29 (14 females and 15 males) affected individuals. Male to male transmission was documented. There was no history of diabetes and/or renal involvement in the family. Histopathological examination of skin lesions showed transepidermal elimination of altered dermal collagen bundles. To the best of our knowledge, the present family is the first and largest in which the disease clearly shows an autosomal dominant mode of inheritance.

Cerebrotendinous xanthomatosis(CTX). The first mexican family reported. *I.A. Gamboa¹, C.F. Salinas², R. Mendivil³, J.M. Aparicio⁴, T.A. Carvajal⁵.* 1) Escuela de Medicina, Benemérita Univ. Autónoma. de Puebla, Puebla, Pue, Mexico; 2) Div. Craniofacial Genetics, Medical University of South Carolina, Charleston, SC; 3) Secretaría de Salud del Edo. de Puebla; 4) Hospital del Niño Poblano, Puebla; 5) Laboratorios Zacatecas, Cd. de Mexico, Mexico.

Cerebrotendinous xanthomatosis (CTX) is a rare inherited lipid-storage disease characterized by progressive neurologic dysfunction, premature atherosclerosis and cataracts. Large deposits of cholesterol and cholestanol are found in every tissue particularly the Achilles tendons, brain and lungs. The onset is in the second decade and it is of autosomal recessive mode of inheritance. The enzyme defect resides in sterol 27-hydroxylase, a mitochondrial cytochrome P-450 enzyme. CYP27 is short for CTX gene. Leitersdorf demonstrated that CYP27 gene contains 9 exons and 8 introns and encompasses at least 18.6 kb of DNA. Gene Map locus: 2q33qter. In Almolya, a small community near the city of Puebla, Mexico, we found a consanguineous family with two affected sisters of 28 and 23 years of age. Six other siblings were normal for CTX. The affected women had short stature, and showed xanthomas of the Achilles tendons and mild mental retardation. Both were treated for bilateral cataracts and are under simvastatine treatment to lower their high cholesterol levels with good results. Both have history of chronic diarrhea. The oldest women became pregnant in her twenties and a girl was born with low birth weight. She now shows short stature, peculiar face with telecanthus, low set and prominent ears and mental retardation. This case does not show additional stigmata, however clinical findings of CTX are commonly observed after puberty. The last case raises the question of whether she is a CTX carrier or is an affected individual. This is of importance for clinical delineation as well as for treatment considerations in order to at least arrest the progression of the disease. To our knowledge this is the first familial case reported in Mexico and also in a family with known native Mexican Indian ethnic background.

Hennekam syndrome. Report of a case diagnosed at birth. *C. Arioni¹, C. Bellini¹, D. Massocco¹, C. Campisi², G. Taddei³, G. Serra¹.* 1) Dipartimento di Pediatria, Università di Genova, Dipartimento Pediatria (DiPe), Istituto Gaslini Genova, Italia; 2) Dipartimento di Scienze Chirurgiche Anestesiologiche e Trapianti d'Organo (DiSCAT), Università di Genova, Italia; 3) Dipartimento di Medicina Interna (DiMi), Università di Genova, Italia.

CASE REPORT. The patient was born prematurely to healthy and unrelated parents. Physical examination revealed a flat face, a depressed and broad nasal bridge, a bulbous nasal tip, mild down-slanting palpebral fissures, epicanthal folds, puffy eye lids, hypertelorism, small mouth and low set ears. The skin appeared marbled and the fingertips were prominent. Generalized lymphedema was especially severe in the periorbital region, on the lower arms and hands, on the lower legs and both feet, and on the penis and scrotum. Hypoproteinemia, hypogammaglobulinemia, and lymphatic count approx 5000 mmc were present. Two subsequent examinations of alpha-1-antitrypsin fecal excretion showed 331 and 3800 gamma / gr of wet feces (normal value 7.7-159 gamma / gr of wet feces), respectively, suggesting intestinal lymphangiectasia. His parents refused to consent to duodenal biopsy. Radionuclide scintigraphy (0.32 mCi of 99mTc-Lymphoscint) showed delayed drainage of the contrast liquid was observed, and was especially evident in the right inguinal area. Chromosomes, TORCH titer, and skull films were normal. Ultrasound evaluation was normal, a part from mild ascites. He did not show any symptoms pointing to psychomotor delay. In 1989, Hennekam et al. first reported a new autosomal recessive syndrome (OMIM 235510) characterized by the presence of lymphedema of the limbs, facial anomalies, intestinal lymphangiectasia, and/or varying degrees of mental retardation. It could be hypothesized that during fetal life, maternal support compensates for the possible intestinal effect of lymphatic vessel abnormalities, which later lead to growth retardation. Some differences in the clinical picture that were seen in our patient may be related to the early age at observation. Additional reports of neonatal cases and full follow-up studies would better delineate the clinical course of the syndrome.

X-Linked syndrome of diarrhea, polyendocrinopathy, and fatal infection in two male monozygotic twins. *M. Mazzella, I. Bertini, C. Arioni, D. Massocco, P. Mezzano, C. Bellini, G. Serra.* Dipartimento Pediatria, Università di Genova, Istituto Gaslini, Genova, Italia.

CASE REPORT. We report on two male monozygotic twins who present neonatal diabetes, intractable diarrhea, immune dysregulation and polyendocrinopathy. They were born after 36-week uncomplicated pregnancy to unrelated healthy parents. Family history was negative. Both patients were admitted because of poor feeding, dehydration, lethargy, metabolic acidosis, severe watery diarrhea and persistent hyperglycemia (up 17 mmol/L; C-peptide absent). High levels of thyroid stimulating hormone, increased serum level of antithyroglobulin and increased antithyroid microsomal antibodies. ICA and GAD-reactive autoantibody were present. Serum enterocytes, anti-parietal cell, and antiendomysium autoantibodies were negative (maternal serum studies failed to demonstrate the presence of autoantibodies). Lymphocytes subpopulations, CD4:CD8 ratio, mitogenic responses did not reveal immunological defects. TORCH serology and IgE concentration were normal. HLA typing did not show type 1 Diabetes related haplotypes in both the patients. Persistent watery diarrhea, failure to thrive, recurrent infections, brittle diabetes characterized the clinical course. Both patients died within the third month of life. Histological evaluation showed marked mononuclear infiltration in the pancreas, gut, and thyroid. Our patients are affected by a very rare X-linked autoimmune enteropathy with polyendocrinopathy. Our cases are interesting because of the presence of neonatal diabetes with histological and immunological findings that are typical of type I diabetes but with the absence of typical HLA antigen pattern of susceptibility. The histological and immunological findings seem to be typical of the autoimmune-type diabetes; the striking finding is that the insulinitis can be clinically evident even shortly after the birth. Neonatal diabetes mellitus is a rare illness. Congenital insulin-dependent diabetes mellitus presenting in the first days of life, is even more rare. Secretory diarrhea unrelated to infections is also very uncommon in infants.

Cleft sternum and aplasia cutis. *E. Zullino, F. Campone, D. Massocco, C. Bellini, G. Serra.* Dipartimento Pediatria, Università di Genova, Istituto Gaslini, Genova, Italia.

Case Report. A female twin infant was born at term to healthy non-consanguineous parents. The twin brother was healthy. No maternal abuse of alcohol, smoke or drug were reported. The child suffered asphyxia at birth and she underwent cardiopulmonary resuscitation. Thoracic X-ray examination showed superior sternal cleft (approx two-thirds). An isolated small area (0.8 cm in diameter) of aplasia cutis on the anterior trunk was observed. US evaluation confirmed the lesion. Brain and cardiac US were normal. No other skeletal defect was observed. Cleft sternum is a rare thoracic defect due to an embryonic fusion failure during the sixth week after conception. The sternum originates from the lateral plate mesoderm and forms two laterally located mesenchymal bands at the sixth week after conception. Both bands fuse during the ninth week in the cranio-caudal fashion. At the end of the eighth week a mesenchymal infiltration stabilizes the overlying ectodermal epithelium. Superior clefts are most often isolated, or associated with vascular dysplasias. Inferior or complete sternal clefts are usually associated with various other defects of the same developmental field. Associated skin anomalies include facial angiomatosis, a supraumbilical midline raphe, a skin ulcer, keloid, or skin defect. The cause of sternal cleft is unknown. All observations of sternal clefts and/or supraumbilical raphe have been sporadic. No teratogen has been recognized in the literature. It is unlikely that nutritional factors play a role in the pathogenesis of cleft sternum; in fact, most reported patients were born in well-developed industrial countries. Arguments for or against a specific genetic origin are generally lacking. An autosomal recessive inheritance has been suggested, but not confirmed. A female twin (zygosity unknown) with superior cleft sternum associated with supraumbilical raphe, and hemangioma of the face and of the neck has been reported. Our case is a further report of this rare malformation. The existence of a healthy twin-brother could confirm that the teratogenic origin is unlikely.

A Family with Cleft Palate - Lateral Synechia Syndrome: Report on Additional Findings and Evidence for Intrafamilial Variability. *L. Fernandez*^{1,2}, *J.C. Prieto*^{1,3}, *O.L. Gutierrez*³, *R. Romero*³. 1) Instituto de Genetica Humana, Universidad Javeriana., Bogota, Cundinamarca, Colombia; 2) Universidad del Rosario, Bogota, Colombia; 3) Hospital la Victoria, Secretaria Distrital de Salud, Bogota, Colombia.

Cleft palate - lateral synechia syndrome (CPLS) is a rare disease characterized by the presence of multiple cordlike adhesions between the free borders of the palate and lateral parts of the tongue and floor of the mouth. It has an autosomal dominant pattern of inheritance. In addition to cleft palate and lateral synechia, other findings such as synechia fused eyelids, blocked or absent nasolacrimal duct, blepharophimosis, convex or beaked profile of nose, full cheeks, trismus, pits of lower lip, prominent or everted lower lip, short philtrum, cleft upper lip, cleft uvula, absent or hypoplastic thumbs, club foot, mental retardation and developmental delay have been associated with this syndrome. Here we report a family with additional phenotypic manifestations mainly in genitalia and limbs. Some of this findings are rectum - vagina fistula, clinodactyly of fifth finger, hypoplasia of midphalanx of fifth finger, short fifth finger, absence of the longitudinal arch of feet and short fingers of the hands. We present this case of CPLS syndrome with three affected family members in which is demonstrated the autosomal dominant inheritance pattern and intrafamilial phenotypic variability. To our knowledge, no other reports have described patients with this syndrome and a rectum - vagina fistula and abnormalities of limbs.

Spinocerebellar Ataxia, Hypogonadotropic Hypogonadism, High Myopic Retinal Degeneration, and Mental Retardation in a 32-Year-Old Man. *R.S. Colby, L.H. Seaver.* Greenwood Genetic Ctr, Greenwood, SC.

A 32-year-old Caucasian male with ataxia and mental retardation (MR) was evaluated. Family history was negative. Perinatal histories were unremarkable. Development was considered normal until 1 year of age when he could not walk alone. Thereafter, his development was delayed and IQ testing showed functioning in the moderate to severe range of MR. Examination showed a eunuchoid habitus and a high pitched voice. He had only light blond facial hair, sparse axillary hair, and pubic hair in a female distribution. Neurologic exam showed horizontal nystagmus, head titubation, action tremor, poor finger to nose and heel to shin testing, and 3-4+ reflexes bilaterally in all limbs. Eye exam showed high myopic retinal degeneration. MRI of the brain showed cerebellar, pontine, and brainstem atrophy. Serum LH and FSH were significantly low. EEG, EMG, and motor and sensory nerve conduction studies were normal. Chromosomes, methylation at the Prader-Willie/Angelman locus, serum lactate, serum transferrin isoelectric focusing, and plasma amino acids were normal.

The association of spinocerebellar ataxia (SCA) and hypogonadotropic hypogonadism (HH) has been noted for some time (OMIM *212840). The added feature of chorioretinal dystrophy is the so-called Boucher-Neuhauser syndrome (BNS)(OMIM *215470). Patients with BNS typically present in adulthood and do not have MR. Laurence-Moon syndrome features include retinitis pigmentosa, MR, spastic paraplegia, and hypogonadism, and may be considered in the differential diagnosis. This patient has high myopic retinal degeneration, but no chorioretinal dystrophy at this point. He also presented at a younger age than most previously reported patients and has MR. Patients with the association of SCA, HH, and retinal changes have shown evidence of autosomal recessive inheritance. X-linked inheritance cannot be excluded in this patient as there was no consanguinity or other siblings affected. This patient seems to fit into the general category of SCA and HH, with or without MR and retinal changes. The possibility that these disorders represent a spectrum seems plausible.

A fourth patient with the Kapur-Toriello syndrome: Pancreatic nesidioblastosis, digital hypoplasia and late paternal age as novel findings. *G.E. Graham¹, A. Pinto², R.B. Lowry¹.* 1) Department of Medical Genetics; 2) Department of Pathology, Alberta Children's Hospital and University of Calgary, Calgary, Alberta, Canada.

In 1991 Kapur and Toriello described a brother and sister with severe intellectual handicap, growth retardation, similar facial features (a bulbous nasal tip and long columella), cleft lip, cleft palate, congenital heart defects, intestinal anomalies and minor digital anomalies. Eight years later, a third patient described by Zelante et al. confirmed the existence of this new and presumably rare MCA/MR syndrome. We now report a fourth patient in whom we suspect Kapur-Toriello syndrome (KTS). This male infant was born at term to a 24-year-old non-diabetic primigravida and her unrelated 50-year-old partner. Our patient had microbrachycephaly, a large anterior fontanelle, iris and retinal colobomata, severe unilateral cleft lip, cleft palate, square-shaped helices, a short neck, excess nuchal skin, PDA, single umbilical artery, short 5th fingers with narrow nails, hypoplastic fourth toes and absent 4th and 5th toenails. He had a complicated and protracted medical course that included growth retardation, severe hyperinsulinemic hypoglycemia and sepsis, to which he succumbed at 6 months of life. An autopsy revealed hypertrophy and hyperplasia of the pancreatic islet cells associated with multifocal ductoinsular structures, consistent with pancreatic nesidioblastosis. Metabolic investigations, as well as amniocyte and lymphocyte chromosomes, were normal. Our patient provides further confirmation of the existence of this MCA/MR syndrome and suggests that pancreatic nesidioblastosis can be one of the intestinal manifestations. We recommend that newborns with features of KTS be closely monitored for hypoglycemia. KTS may represent a microdeletion syndrome or a single gene disorder caused by an autosomal recessive or new dominant mutation. It may also be etiologically heterogeneous. Advanced paternal age would support a new dominant mutation in our patient, but other modes of inheritance cannot be ruled out.

Clinical data and Molecular Analysis in twenty-four Mexican Apert syndrome patients. *L. Cornejo*¹, *E. Garca*², *S. Chavira*³, *M. Muenke*⁴, *D. Saavedra*³. 1) School of Medicine, Sciences Health Institute, Autonomous Hidalgo State University, Pachuca de Soto, Hidalgo Mexico; 2) West Biological Research Center and Mexican Social Security Institute, CIBO-IMSS, Guadalajara, Jalisco Mexico; 3) General Hospital "Dr. Manuel Gea Gonzalez" SSa, Mexico City, Mexico; 4) Clinical Genetics Branch, NHGRI, NHI, Bethesda Ma, USA.

Introduction.- Apert syndrome is characterized by craniosinostosis, midface hypoplasia, and symmetric syndactily of the hands and feet, minimally involving digits 2,3, and 4. The syndrome has been observed in white, black, and Asian populations. Its inheritance pattern is Autosomal Dominant (AD). Molecular analysis has reported mutations in *FGFR2* gene as: Ser252Trp, Pro253Arg, Pro253Phe; acceptor splice site and *Alu* insertion in patients with Apert syndrome worldwide. **Material and Methods.-** A clinical re-evaluation in 24 mexican patients (mixture of Asiatic and Iberic genes) out of 79 was done. All of them have been attended at the General Hospital "Dr. Manuel Gea Gonzalez" since fifteen years ago. Also the sequencing of exon 7 in *FGFR2* gene was done. **Results.-** Twenty three patients have a heterozygous allele mutation and only one patient has a homozygous allele mutation. Thirteen patients showed a Serine252Trp (TCG to TCC) mutation (homozygous allele);and eleven patients showed a Pro253Arg (CCT to CGT) mutation. Clinical data were not different from the previously worldwide reported cases, including the patient with a homozygous allele mutation. **Conclusions.-** This is the first Mexican - meztizo population series with Apert syndrome reported with clinical and molecular data. The patients show clinical and molecular similarity, included craniofacial antropological measurements. Homozygous allele mutation is not a more deletereous or lethal than heterozygous condition as in other AD diseases.

Monozygotic twins with fetal akinesia: The value of clinico-pathological work-up in predicting recurrence risks.

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Fetal Akinesia Deformation Sequence (FADS) or Pena-Shokeir Sequence is a heterogeneous group of disorders in which prolonged decrease or absence of fetal movements results in a series of deformational anomalies: multiple contractures, pulmonary hypoplasia, craniofacial anomalies, polyhydramnios, intrauterine growth retardation, and short umbilical cord. Three sets of monozygotic twins, two pairs concordant for FADS and one set discordant, were presented, and the clinical and pathological findings were reviewed. Detailed pathological work-up established that the two pairs of twins concordant for FADS were of myogenic etiology while the set discordant was due to anoxic-ischemic damage. Much insight into FADS can be gained from the pathological findings of two studies on arthrogryposis multiplex congenita (AMC) (Strehl et al., 1985; Quinn et al., 1991). Like FADS, AMC is not in itself a diagnosis but a descriptive term, comprising two or more joint contractures since birth, and is associated with any factors that interferes with fetal movements. In the first series of 22 cases of AMC, 9 cases were determined by Strehl and co-workers to be myopathic in etiology. In the other series of 21 cases analysed by Quinn et al., 11 cases were ascertained to be of myogenic origin. A high rate of recurrences was also noted in the myogenic group. This study on monozygotic twins with fetal akinesia focussed on how the etiological findings resulting in FADS could help in predicting risks of recurrence.

Bilateral coronal craniosynostosis in a patient with Opitz syndrome. *J. Hedrick, D. Waggoner.* Dept. of Human Genetics, University of Chicago, Chicago, IL.

Opitz (G/BBB) is a syndrome of multiple congenital anomalies characterized by hypertelorism and hypospadias as well as a number of abnormalities of midline structures, including cleft lip and palate, laryngotracheoesophageal defects, imperforated anus, brain anomalies, congenital heart defects, and renal anomalies. Other common findings in Opitz syndrome include downslanting palpebral fissures, epicanthal folds, micrognathia and developmental delay/mental retardation. Opitz syndrome is a heterogeneous condition with loci at 22q11 and Xp22 resulting in both autosomal dominant and X-linked inheritance. Deletions of the well known DiGeorge and Velocardiofacial syndromes (DGS/VCFS) critical region at 22q11 and various mutations in the MID1 gene at Xp22 have been detected in a number of patients with a clinical diagnosis of Opitz syndrome. There is no apparent phenotypic difference between these two groups of individuals. While cranial asymmetry has been associated with Opitz syndrome, craniosynostosis has only been reported once. We report the second case of craniosynostosis and Opitz syndrome in a 9-year-old male ascertained through our multidisciplinary craniofacial clinic. He has a history of developmental delay, bilateral cleft lip and palate, hypertelorism, downslanting palpebral fissures, micrognathia, bilateral coronal craniosynostosis, hypospadias, and premature narrowing of the airway. Chromosome analysis and FISH with TUPLE1 were both normal. Another disorder, Craniofrontonasal syndrome (CFNS), a rare genetic condition characterized by coronal craniosynostosis and a number of features similar to Opitz syndrome with the exception of hypospadias and laryngotracheoesophageal defects, also localizes to Xp22. Due to low copy repeats distributed throughout this region, it is possible that the X chromosome may be more susceptible to unbalanced rearrangements resulting in a microdeletion causing the overlapping phenotype of Opitz syndrome and CFNS in this patient. Molecular analysis of this region is being pursued.

Further exploring the relationship between lupus and chondrodysplasia punctata. *D. Kostiner, J. Derstine.* Dept. Pediatrics/Division of Genetics, University of California, San Francisco, CA.

There are several reports in the literature of babies with chondrodysplasia punctata being born to mothers with systemic lupus erythematosus (SLE). The clinical manifestations are similar to those seen with prenatal exposure to oral anticoagulants. It is suspected that maternal autoantibodies produce the spectrum of clinical features. We present the case of a 25-year-old Chinese woman with facial manifestations suggestive of chondrodysplasia punctata who developed lupus as a teenager. We have little information about her parents, but consanguinity is suspected. It is not known whether the patient's mother had lupus. If she did, we could speculate that maternal lupus during pregnancy caused the craniofacial abnormalities in the patient, and that inheritance of a certain HLA type predisposed the patient to developing SLE herself. The patient was born at term after an uncomplicated pregnancy. Birth weight was 3700 gm (60%), length was 19 in (25%), HC was 33 cm (<3%; 50% for 34 weeks). Significant hypotonia was noted in the newborn period. Developmental milestones were delayed. SLE, primarily discoid lupus, was diagnosed at age 16 years. Left hip replacement was performed at age 16 years for recurrent hip dislocations. Craniofacial surgery was performed at age 20 years to correct a hypoplastic mandible. The patient has myopia and bilateral sensorineural deafness. She has mental retardation, lives with a guardian and attends school. Current exam reveals the following: Ht 152 cm (<3%), Wt 51.3 Kg (25%), HC 52.5 cm (-1.8 SD). Dysmorphic facies. Low hairline. Synophrys. Midface hypoplasia. Large eyes. Proptosis. Hypertelorism. Simple ears. Hypoplastic nasal bridge. Flat nasal tip. Paucity of bone and cartilage in nose. Hypoplastic mandible, status-post surgical repair. Mild hepatomegaly. Short digits. No obvious rhizomelia. Abnormal skin and nails secondary to lupus. Chromosomes, peroxisomal studies, and skeletal survey are currently pending. In summary, documentation of SLE in the patient's mother could account both for features of chondrodysplasia punctata in the patient as well as autosomal dominant predisposition to development of SLE.

Megalencephaly-Cutis Marmorata Telangiectatica Congenita Syndrome: Association of arrhythmia leading to sudden death? *S. Yano*^{1,2}, *Y. Watanabe*^{2,3}. 1) Div Medical Genetics, Childrens Hosp, Los Angeles and University of Southern California, Los Angeles, CA; 2) Medical Genetics, Pediatrics, Kurume University, Kurume Japan; 3) Pediatrics, University of Iowa.

Megalencephaly-cutis marmorata telangiectatica congenita (M-CMTC) constitutes a distinct entity characterized by prenatal overgrowth, macrosomia, hemihypertrophy, macrocephaly, nonobstructive hydrocephaly, frontal bossing, hypotonia, developmental delay, generalized or facial capillary malformation with upper philtral nevus flammeus and cutis marmorata, joint hypermobility, loose skin, toe syndactyly, and postaxial polydactyly. All but one of the cases reported previously had benign clinical courses without showing an increased risk of early infantile death. We describe three additional cases with poor clinical outcomes including severe postnatal growth failure, intractable cardiac arrhythmia in two cases and sudden infant death in two cases. Arrhythmia has not been described previously as one of the symptoms of M-CMTC. Patients with M-CMTC associated with severe postnatal growth failure and arrhythmia may constitute a distinct clinical subtype of M-CMTC with an increased risk of life-threatening episodes or sudden death. Recognizing this clinical subtype of M-CMTC is important in order to prevent these serious potential complications.

FGFR3 P250R Mutation Associated with Hemifacial Microsomia, Craniosynostosis and Sudden Infant Death. K.

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FGFR3 P250R mutation is an autosomal dominant, mostly familial condition associated with coronal craniosynostosis, sensorineural deafness, craniofacial and digital abnormalities. We report a mother and daughter with this mutation. The mother presented with coronal craniosynostosis and hemifacial microsomia and her daughter died unexpectedly of upper airway obstruction following which a genetic investigation revealed that both carry the P250R mutation. The G₁P₁L₀ mother was seen after the unexpected death of her 2-day old daughter. At birth the infant required oxygen for tachypnea and noisy breathing was noted without cyanosis or apnea. The day after discharge she was found unresponsive. The post-mortem showed dysmorphic features including down-slanting palpebral fissures, left proptosis, preauricular skin tags, right coronal craniosynostosis and orbital hypoplasia. Examination of mother revealed brachycephaly, ridging of coronal sutures, aplasia of the right external auditory meatus, mild proptosis, malar hypoplasia, and bilateral brachydactyly of 5th fingers and 1st toes, and syndactyly of 2nd and 3rd toes. In infancy a diagnosis of Treacher Collins syndrome had been made. In childhood X-rays had shown bicoronal craniosynostosis, bony atresia of the external auditory canal and malar hypoplasia; and osteotomies had been performed for bilateral varus metatarsus deformities.

Molecular testing confirmed the presence of FGFR3 P250R mutation in both mother and deceased infant. Though unavailable for testing the grandfather was reported to have similar facial features to his daughter and deceased granddaughter. These patients add hemifacial microsomia and sudden infant death to the clinical manifestations of the P250R mutation. It again emphasizes the inter- and intra-familial variability of manifestations in this condition and the increased severity in females reported previously.

Linear Nevus Sebaceus with Empty Sella Turcica and Transsphenoidal Meningocele. *J.C. Prieto^{1,2}, A. Motta³, L. Fernandez⁴, R. Romero², O.L. Gutierrez², P. Garavito⁵.* 1) Instituto de Genetica Humana, Universidad Javeriana, Bogota, Colombia; 2) Hospital la Victoria, Secretaria de Salud, Bogota, Colombia; 3) Hospital Simon Bolivar, Secretaria de Salud, Bogota, Colombia; 4) Universidad del Rosario, Bogota, Colombia; 5) Universidad del Norte, Barranquilla, Colombia.

Linear sebaceous nevus syndrome also known as nevus sebaceus of Jadassohn is characterized by the presence at birth or soon after, of alopecia with absent or primitive hair follicles and numerous small hypoplastic sebaceous glands with hyperpigmentation and hyperkeratosis. Lesions are usually on the scalp, in the para - midfacial area, from the forehead down into the nasal area. These tend to be linear in distribution, and may also affect the trunk and limbs. At puberty, the lesions become verrucous with hyperplastic sebaceous glands, and tumors may develop. Other findings can include asymmetric overgrowth, cranial asymmetry, lid lipodermoid, coloboma of eyelids, iris and choroid, coarctation of aorta, horseshoe kidney, kyphoscoliosis, finger and toe abnormalities, hemangioma, mental retardation, seizures, vitamin D resistant rickets basal cell carcinoma, epidermal nevus, hydrocephaly, ventricular septal defect, hypoplastic teeth, renal hamartomata and nephroblastoma. The cases reported have been sporadic, a specific pattern of inheritance has not been described, yet. We report on a 26 years old man with bilateral ptosis, amaurosis of the right eye, ocular hypertelorism, telecanthus, broad nasal bridge, hypoplastic nostrils, kyphoscoliosis, gynecomastia, sexual infantilism, absence of testes, bilateral hallux valgus, clinodactyly of fifth finger, bilateral limitations for elbow extension and linear verrucous nevus on the lower extremities. The hormone studies reported a hypopituitarism. The cytogenetic analysis was normal 46, XY. The brain MRI showed a transsphenoidal meningocele which extends to the nasalpharyngeal tract; traction of the optic nerves and optic chiasm and there is not evidence of the hypophysis. This new case of linear nevus sebaceus contributes with new clinical and radiological findings that have not been reported on previous cases.

40 years later: The health related quality of life of women affected by Thalidomide. *I. Nippert.* Institut fuer Humangenetik, Westf. Wilhelms-Universitaet, Muenster, Germany.

Introduction: Almost 40 years ago the sudden rise of births of babies with a range of severe deformities shocked the world. It was caused by a drug: the tranquilizer Thalidomide. Thalidomide was marketed in more than 40 countries worldwide. Pregnant women who took the drug in the first trimester of their pregnancy had children with a wide but distinctive range of malformations. Thalidomide claimed more than 10.000 victims in Australia, the U.S.A., Germany, Japan, Sweden, the U.K., etc. Those who survived are now reaching middle-age. How have they fared in major areas of life such as education, employment, reproduction and how is their health related quality of life? Especially women affected by Thalidomide may be at special disadvantage because of combined discrimination based on gender and disability.

Methods: A questionnaire using the WHO QOL-BREF instrument for measuring the health related quality of life was distributed among 166 females affected by Thalidomide, living in the state of North-Rhine Westphalia, Germany. A randomized control group matched by age and area of residence was asked to fill out a corresponding questionnaire. The response rate was 62%.

Results: Women affected by Thalidomide have a significantly lower overall health related quality of life score (63.01) as compared to their matched controls (70.71). They were found to have a marked lower physical health score (67.87) as compared to the controls (77.82). They are significantly less frequently married, have fewer children, have less household income and are less mobile. They suffer from chronic pain because of a steady progress of deterioration of bones and muscles. They rely heavily on medical treatment and are less satisfied than their controls with the availability of health services that meet their needs. Because of ongoing gradual impairment they face an uncertain future in regard to their mobility and their ability to remain in the workforce.

Autosomal dominant primary empty sella syndrome, congenital glaucoma, sensorineural hearing loss, and tooth dysgenesis. *S. Finzi¹, J. Auguste¹, E.A. DelBono¹, J.L. Haines², J.L. Wiggs^{1,3}.* 1) Ophthalmology, New England Medical Center, Boston, MA; 2) Program in Human Genetics, Vanderbilt School of Medicine, Nashville, TN; 3) Division of Clinical Genetics, New England Medical Center, Boston, MA.

Primary empty sella syndrome results from a defect in the diaphragm of the pituitary sella that causes the subarachnoid space to extend into the sella turcica. Inherited forms of this condition have been previously associated with osteosclerotic dysplasia and cranio-facial deformities. A gene responsible for this condition has not been identified, although two patients with primary empty sella syndrome, mental retardation and skeletal abnormalities have been found to have a tandem duplication of Xq13-q21. We have identified a three generation pedigree with 11 affected individuals demonstrating the novel association of primary empty sella syndrome with ocular defects, sensorineural hearing loss and tooth abnormalities. Several affected individuals also have a flat mid-face and a high-arched palate. The ocular defects include: congenital glaucoma, posterior embryotoxon, and lens coloboma. The hearing loss develops in the second to third decades and is associated with periodic vertigo characteristic of Menieres disease. Tooth abnormalities include: delayed secondary dentition, malformed incisors, and extra teeth. Enlargement of the sella turcica was determined by MRI. There was no indication of hydrocephalus or pituitary dysfunction in any of the family members with sella turcica enlargement. Affected individuals are present in three consecutive generations, and equal numbers of males and females are affected. Male to male transmission supports autosomal dominant inheritance. Cytogenetic analysis did not reveal any deletions, insertions, inversions or translocations. Genetic linkage studies using markers flanking existing loci for congenital glaucoma or Axenfeld-Rieger syndrome indicate that this pedigree is not linked to any of these previously identified regions. A genome screen designed to determine the location of a gene responsible for this interesting syndrome is currently underway.

Microgastria In The Genetic Clinic. *B.G. Kousseff.* Division of Medical Genetics, Department of Pediatrics, University of South Florida, Tampa, 33613, (813) 233-2720.

Microgastria is a rare anomaly of the GI tract. It is a solitary malformation or part of multiple anomalies (syndromic microgastria). Prenatally absent/tiny stomach bubble is the sonographic sign. Between January 2, 1982 and April 15, 1999 through the USF Genetic clinics there were ten probands with suspected/confirmed microgastria. They were part of the 35,414 probands/families evaluated. Four were seen through the pediatric genetic clinic. All had microgastria with associated anomalies: JC a Mexican one-year-old had it with extensive partial albinism, mixed bilateral hearing loss, blepharophimosis, abnormal nasolacrimal ducts, ASD, pulmonic stenosis, pectus carinatum, asymmetric brachydactyly, mild mental retardation and 46, XY karyotype. JH was a newborn Caucasian girl with polyhydramnios, IUGR, TE fistula, tetralogy of Fallot, partial situs inversus, second and third toe syndactyly and 46, XX karyotype. CC was a premature newborn Caucasian girl with polyhydramnios, TE fistula, spleen with extraneous lobe, GE reflux, pectus carinatum and left inguinal hernia. DW was a newborn Caucasian boy with left cleft lip and palate, GE reflux, ectrodactyly, failure to thrive, short stature, and 46, XY karyotype. All required small feedings and DW has surgery to create a larger stomach. PS, LM, MM, RC, SS, CS were seen through the prenatal clinic. On targeted sonograms their fetuses did not show a "stomach bubble." The male fetus of PS had trisomy 21 with single umbilical artery and polyhydramnios; a stillbirth took place (PS developed class A1 diabetes mellitus during the gestation). LM had a stillborn girl with anencephaly, sacral spina bifida and cleft lip. Autopsies of the stillborns were declined. The 46, XX fetus of MM showed also single umbilical artery and polyhydramnios. The fetuses of RC, SS and CS did not show other abnormalities but all failed to return to the clinic. Postnatal genetic evaluations are a must whenever microgastria is suspected. Without them "no stomach bubble" may or may not represent microgastria. The "absent bubble" is a sonographic impression which requires confirmation. Otherwise microgastria is over-diagnosed.

Eye findings in Prader-Willi syndrome. *R. Fox¹, R.B. Sinatra¹, M.A. Mooney¹, G.S. Yang¹, I.D. Feurer¹, T. Thompson¹, M.G. Butler².* 1) Psychology, Vanderbilt University, Nashville, TN; 2) Children's Mercy Hospital, Kansas City, MO.

Prader-Willi syndrome (PWS) is characterized by infantile hypotonia, obesity in childhood, small hands and feet, hypogonadism, mental retardation, and a paternally derived 15q11-q13 deletion or maternal disomy 15. We report on ophthalmology and visual capacity studies in 43 PWS subjects (24 with 15q11-q13 deletion and 19 with maternal disomy 15), 22 non-PWS equivalent controls and 14 normal controls. No significant differences were found between the deletion or maternal disomy subgroups for standard ophthalmic measures. Similar eye findings were present in the control group except for myopia [larger refractive errors in the PWS group (mean = -5.56 for PWS and mean = -2.33 for equivalent controls)] and stereopsis (81% of controls had stereopsis by performance tests compared with only 44% of PWS subjects). An effect of genetic subgroup was observed for random element stereopsis with maternal disomy group having a greater degree of impairment. Additionally, discrimination of shape of motion produced by forms generated from random elements displayed on a monitor screen was tested in the four subject groups: PWS chromosome 15q deletion subtype; PWS maternal disomy; non-PWS equivalent controls; and normal controls. The performance of the normal controls exceeded all other groups (78% with correct responses in identifying moving forms generated by random elements displayed on the monitor screen, $p < .009$). The PWS deletion group (66% with correct responses) and the equivalent control group (59% with correct responses) did not differ ($p = .95$). However, the PWS maternal disomy group performed significantly worse (38% with correct responses, $p < .004$) than all other groups. The performance of the PWS deletion subjects and equivalent controls is consistent with other data indicating these populations encounter difficulty meeting the processing demands posed by second order visual stimuli. The inferior performance of the maternal disomy group may be due to two active alleles of a maternally expressed gene influencing neural development. One such candidate is the UBE3A gene which is maternally expressed and localized to the 15q11-q13 region.

Phenotypic Characteristics of Hereditary Benign Intraepithelial Dyskeratosis (HBID). *P. Challa¹, G.K. Klintworth¹, B. Seo², M. Bembe², E. Rampersaud², T. Parrish², J. Gilbert², M.A. Pericak-Vance², J.M. Vance², R.R. Allingham¹.* 1) Department of Ophthalmology, Duke University Medical Center, Durham, NC; 2) Center of Human Genetics, Duke University Medical Center.

Hereditary benign intraepithelial dyskeratosis (HBID) is an autosomal dominant disorder of the ocular and oral mucous membranes first described in 1959 by Von Sallman and Paton. The condition predominantly affects a Native American population in North Carolina. HBID has been recently localized to a small duplicated region at the 4q35 locus (see ASHG, Vance et al). HBID generally presents before age 5 years. Individuals present with bilateral conjunctival mucosal plaques and associated erythema. The ocular injection characteristic of HBID has caused the local population to refer to it as "red eye disease". Individuals often complain of irritation and redness but rarely require surgery. Asymptomatic oral buccal lesions are also seen in individuals with HBID. Lesions apparently do not undergo malignant transformation. Provided here is a comprehensive phenotypic description of HBID based on the individuals examined by the authors. Informed consent was obtained from all available members of two families with HBID. Complete ocular and oral exams were performed on fifty-five individuals. Of the twenty-five individuals who had the duplication at the 4q35 locus, all had bilateral conjunctival involvement with variable plaque formation from a translucent granular appearance to nodular whitish lesions with deep and superficial vascularization. Erythema was variable from mild to severe with most in the moderate range. Visual acuity limitation was rare with few individuals requiring conjunctival or corneal surgery. Onset of disease was reported from birth to two years of age in most cases. A few individuals self-reported progressive lesions. Most had associated buccal lesions; of those nearly one-third had focal lingual leukoplakic lesions. A few members reported a seasonal variation in the severity of the conjunctival lesions, with spring and summer being the worst. Individuals who lacked the duplication on 4q35 did not have any of the clinical findings associated with HBID.

Physical and psychological development of individuals prenatally diagnosed with 45,X/46,XX. *S.A. O'Neill¹, K. Ghelani², J. Rovet², D. Chitayat¹.* 1) Division of Clinical Genetics, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; 2) Department of Psychology, The Hospital for Sick Children, Toronto, Canada.

Introduction: Although several recent reports have indicated that parents and health care professionals request information about the prognosis for patients with 45,X/46,XX, information on the long-term physical and psychological development of these individuals is limited. In the present study, we report on the physical and psychological development of 7 children who were diagnosed prenatally with 45,X/46,XX.

Methods: Each child underwent a physical examination and a comprehensive neuropsychological evaluation using selective subtests from the Stanford-Binet Intelligence Scale-Fourth edition, Neuropsychological Investigation for Children, Wechsler Individual Achievement Test, Wide Range Assessment of Visual Motor Abilities, and a Developmental Test of Visual-Motor Integration. As well, the child's behaviour, temperament and social relationships were assessed by the Child Behaviour Checklist, Temperament questionnaire, and Friendship questionnaire. The physical and psychological development of this series of 7 individuals was compared to other individuals with Turner syndrome reported in the literature.

Results: Results from this study show those individuals with mosaicism for Turner syndrome have fewer physical and psychological manifestations than individuals who are non-mosaic. Similarly, individuals diagnosed prenatally with 45,X/46,XX have a milder phenotype than individuals with mosaicism who are postnatally diagnosed.

Conclusions: This study supports the view that individuals prenatally diagnosed with mosaicism for Turner syndrome have a milder phenotype both in physical and psychological development than individuals who are postnatally diagnosed or who are non-mosaic. As this study had a small number of participants, further research to examine the development of girls prenatally diagnosed with mosaicism is needed.

Infantile hypophosphatasia represents compound heterozygosity for a milder autosomal dominant trait. M.E.

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Hypophosphatasia occurs in perinatal, infantile, childhood, and adult forms, the first three displaying autosomal recessive inheritance and the last often displaying autosomal dominant inheritance. We report a family displaying three clinical forms which suggest inheritance of a dominant tissue-nonspecific alkaline phosphatase (TNSALP) gene mutation, haplo-insufficiency explaining the milder phenotype and compound heterozygosity explaining the severe perinatal phenotype. The 15 month-old female proband presented with slow to heal fractures of the radius and metatarsal bones and the diagnosis of osteogenesis imperfecta vs. child abuse. Father of the proband, raised with fluoridated water, has had recurrent dental caries in his 20's. The paternal grandmother lost all her permanent teeth in her 20's. The paternal aunt of the proband died in 1963 at 7 days, according to retrieved autopsy, of "hypophosphatasia with defective ossification of skull and ribs". Serum alkaline phosphatase of the proband was 128 U/l (normal 100-300), of the father 54 U/l (normal 38-126). Complete sequencing of TNSALP (including intron/exon boundaries and untranslated exons) revealed only one mutation, G232V, in both the proband and her father. This specific mutation has previously been reported in conjunction with a second frameshift mutation (compound heterozygote) in a case of perinatal hypophosphatasia, suggesting the recessive mode in the paternal aunt and confirming her clinical diagnosis of 37 years ago. Site-directed mutagenesis and transfection studies are in progress with enzymatic assays of fibroblasts from the proband and her father. This family adds to the growing body of genotype/phenotype correlative evidence suggesting that perinatal and infantile hypophosphatasia arise from compound heterozygosity for severe mutations, and that childhood and adult hypophosphatasia arise from either heterozygosity or compound heterozygosity for severe and/or moderate mutant alleles.

Adaptive functioning and molecular relationships in individuals with fragile X syndrome. *S.W. Harris¹, K.E. Brown¹, J.L. Hills¹, F. Tassone², P.J. Hagerman², A.K. Taylor³, R.J. Hagerman¹.* 1) Fragile X Treatment & Research Center, The Children's Hospital, Denver, CO; 2) Department of Biochemistry and Molecular Genetics, University of Colorado Health Sciences Center, Denver, CO; 3) Kimball Genetics, Denver, CO.

Fragile X syndrome (FXS) is a broad spectrum disorder and, consequently, individuals with FXS demonstrate a variety of cognitive and adaptive abilities. It is useful to measure both IQ and adaptive function in individuals who have FXS, as many individuals may show strengths in adaptive functioning which may not be reflected in their IQ score. Therefore, areas of strength in adaptive functioning may correlate with FMRP levels.

The Vineland Adaptive Behavior Scales includes an Adaptive Behavior Composite (ABC) score, as well as individual domain scores for Communication, Daily Living Skills, and Socialization. These variables, along with percentage of FMRP expression, were utilized for statistical correlation.

We analyzed clinical and molecular data for 86 individuals with the full mutation or mosaic status for FXS, with an age range of 1.6 to 45.5 years. We utilized the Spearman correlation coefficient with a two-tailed t-test and found that the correlation between FMRP and several of the Vineland domains was significant: FMRP and ABC ($p=.002$), FMRP and Communication ($p=.005$), FMRP and Daily Living Skills ($p=.000$). Interestingly, there was no correlation between FMRP and the Socialization domain ($p=.278$).

We also separately analyzed the male group ($n=72$), of which 58 individuals had the full mutation and 14 individuals had a mosaic FMR1 gene, with an age range of 1.6 to 45.5 years. We utilized the Spearman correlation coefficient with a two-tailed t-test and found a significant correlation between FMRP and Adaptive Behavior Composite ($p=.009$), FMRP and Communication ($p=.008$), and FMRP and Daily Living Skills ($p=.004$). And again, the Socialization domain did not correlate with FMRP ($p=.180$), which suggests that this domain is significantly influenced by background gene effects.

A novel mutation in a patient with IGF-I deficiency. *G. Bonapace, D. Concolino, S. Formicola, P. Strisciuglio.*
Department of Pediatrics, Faculty of Medicine, University of Catanzaro. ITALY.

Igf-I is considered to be one of the most important growth factor but its role has not yet made completely clear. A Patient has been recently described, with severe prenatal and post natal growth failure, sensorineural deafness, mental retardation, very low levels of IGF-I, both basal and post generation test, associated with IGF-I gene partial deletion (1). We described a new case with IGF-I deficiency due to a point mutation in exon 4a of IGF-I gene never described before. The patient was born at 39 weeks of uneventfull gestation by cesarean section because of poor fetal growth. The birth weight was 1,840 Kg (-4SD), the lenght was 41 cm (-6,5SD) and the head circumference was of 26,5 cm (<5pc). The parents were second cousins and the family history showed several miscarriages. His mother was 141,5cm (-3SD)tall and his father was 145,7 cm (-4,3SD). At the age of 1,6 years he showed a weight of 4,750 Kg (-6,2SD) the head circumference was 40,5 cm (p<5) and a ratio L/P <5 pc. Mild dysmorphic signs were present. Psicomotor development was delayed; audiograms showed bilateral sensorineural deafness. The basal serum concentration of GH was 1,6 ng/ml and the peak serum after administration of arginine was 18 ng/ml. The basal serum concentration of IGF-I was 23ng/ml (between -3SD and 2 SD) and after the IGF-I generation test, IGF-I levels remained very low (25ng/ml) while IGFBP3 levels did not change. The bone age according to Greulich and Pyle was in neonatal range. The molecular studies performed, showed in our patient a novel mutation (transversion T-A) that destroys the normal consensus sequence for polyadenilation site in the 3' untraslated region of exon 4a of IGF-I gene. Our expression analysis experiments by Rt-pcr demonstrated a reduction in the exon 4a size. In conclusion our results first demonstrated that the transversion on the consensus sequence AAATATA of polyadenilation site of IGF-I exon 4a is the cause of deregulated pattern of mRNA maturation, and therefore of IGF-I deficiency. (1) Woods K.A. et al New Engl.J. Med (1996) 335:1363-1367.

Morphological characterization of non-syndromic Split Hand-Split Foot malformation. *S. Sifakis¹, M. Arici², D. Viljoen³, P. Beighton⁴, P. Tsipouras¹*. 1) Dept of Pediatrics, University of Connecticut Health Center, Farmington,CT; 2) Dept of Radiology, University of Connecticut Health Center, Farmington,CT; 3) Dept of Human Genetics,University of Witwatersrand, Johannesburg, South Africa; 4) Dept of Human Genetics,University of Cape Town, South Africa.

Split Hand-Split Foot malformation (SHFM) describes a developmental defect of the distal extremities characterized by the absence of one or more central rays, and syndactyly. There is considerable phenotypic variability, thus the aim of this study was to identify the presence of common morphological and radiological patterns in cases of non-syndromic SHFM. Clinical information from 16 SHFM cases including 27 radiographs from 39 limbs was retrospectively analyzed. The diagnosis in all cases was unequivocally established to be non-syndromic SHFM. Positive family history was present in 12 of the 16 cases. Six cases were members of the Wadoma tribe residing in the border between Botswana and Zimbabwe. Based on the review of the available radiographs, we were able to distinguish five categories of morphological abnormalities of the hands and feet: 1) Monodactylous limbs associated with aplasia and hypoplasia of a variable number of carpals, metacarpals and phalanges. 2) Lobster-claw anomaly of both hands and feet characterized by deep central clefts and syndactyly. The cleft invariably extended to the base of the metatarsal or tarsal level in the feet, while it did not extend beyond the mid-metacarpal level in the hands. 3) Abnormal articulation of one or more metacarpals or metatarsals to adjacent joints. 4) Abnormal positioning of a transversely oriented long bone between the heads of affected metacarpals and the base of phalanges. 5) Osseous and cutaneous syndactyly. Other findings included triphalangeal thumbs, clinodactyly, and axial polydactyly. In summary, five distinct patterns of skeletal abnormalities were observed in non-syndromic SHFM. The functional effect of the skeletal abnormalities varied from mild to debilitating. This clinical variability represents a phenotypic continuum and possibly reflects the underlying genetic heterogeneity associated with SHFM.

Phenotypic characterisation of three genetically distinct forms of Bardet-Biedl syndrome. *E. Heon^{1,2}, C. Westall¹, R. Carmi³, K. Elbedour's³, C. Panton¹, L. Mackeen¹, E. Stone⁴, V. Sheffield⁴.* 1) Dept Ophthalmology, The Hospital for Sick Children; 2) The Toronto Hospital, University of Toronto, ON, Canada; 3) The Genetic Institute, Ben-Gurion University of the Negev, Beer-Sheva, Israel; 4) Dept Pediatrics, The University of Iowa Hospitals and Clinics, Iowa City, Iowa.

Bardet-Biedl syndrome is an autosomal-recessive multisystemic condition characterised by usually severe retinitis pigmentosa, hypogenitalism, digit anomaly, renal anomalies, obesity, and a variable degree of mental retardation. The disease is known to be genetically heterogenous for which five different loci have been identified on chromosomes 3, 11, 12, 15, and 16. No gene has been identified. Three genetically distinct large Bedouin families from the Negev, affected with Bardet-Biedl syndrome, were examined to define the distinct ocular phenotypes. In addition obligate carriers were studied electrophysiologically to assess if their carrier status could be detected in a reproducible way. Comprehensive eye examination and ERG testing were performed on affected members and obligate carriers available of families linked to chromosomes 3, 15, and 16.

Retinal dystrophy was severe and early in all cases and the three genotypes carried a poor visual prognosis. The major distinctive feature between the 3 groups was the refractive error as one family was very myopic. Also one individual was observed to have bilateral colobomas in addition to the classic features of Bardet-Biedl. Although this finding may be coincidental it may also suggest that Biemond syndrome is either a variant of Bardet-Biedl syndrome or that this may represent a contiguous gene syndrome. It is difficult to detect the carrier status electrophysiologically or clinically in a reproducible way.

Myotonia congenita in the dog. *U. Giger¹, M. Gracis¹, A.L. George Jr.², T.H. Rhodes², C. Fahlke², Y. Rajpurohit¹, D.P. Bhalerao¹, J. Melniczek¹, S.W. Volk¹, D.F. Patterson¹, C.H. Vite¹.* 1) Clinical Studies, School of Veterinary Medicine, University of PA, Philadelphia, PA; 2) Department of Medicine and Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

In the Miniature Schnauzer dog, a mutation in the ClC-1 chloride channel is responsible for autosomal recessive myotonia congenita. The mutation results in replacement of a threonine residue in the D5 transmembrane segment with methionine. The purpose of this study was to characterize myotonia congenita in this recently identified canine model. Eight affected dogs were examined. Affected dogs exhibit a delay in skeletal muscle relaxation following stimulation; diffuse skeletal muscle hypertrophy; stridor; an abnormal bark; and a stiff, stilted gait that improves with exercise. Dental abnormalities including distocclusion, delayed dental eruption of both deciduous and permanent dentitions, delayed dental exfoliation of the primary teeth, and unerupted or partially erupted permanent teeth have been documented. In no other animals affected with myotonia congenita have such dental abnormalities been described. Clinical and electrophysiological signs are first apparent at 5 weeks of age. Carrier dogs showed no clinical or electrophysiological signs of disease. Two affected dogs were treated with procainamide from 4 weeks of age onward; treated dogs showed amelioration of muscle stiffness and of dental abnormalities as adults.

A DNA-based test capable of detecting the mutant allele in both carrier and myotonic Miniature Schnauzers was developed. The incidence of the mutation in the East Coast Miniature Schnauzer population was determined; 30% of 134 dogs tested thus far in a biased sample carry the mutation. All affected dogs examined to date are descendents of a common ancestor.

A breeding colony has been established at the University of Pennsylvania. The availability of a well-characterized canine model of myotonia congenita allows for the study of improved treatments for this disease. Supported in part by NIH (RR02512; NS02032; AR44506; T32-GM07628) and a grant from the Muscular Dystrophy Association.

Heterogeneity in Familial Autosomal Dominant Paget Disease of Bone and Muscular Dystrophy. *B. Waggoner¹, V.E. Kimonis¹, M.J. Kovach¹, D.A. Gelber², R. Khardori³.* 1) Dept. Pediatrics, Division of Genetics and Metabolism, Southern Illinois University, Springfield, IL; 2) Dept. of Neurology, Southern Illinois University, Springfield, IL; 3) Dept. of Internal Medicine, Southern Illinois University, Springfield, IL.

Autosomal dominant muscular dystrophy with the association of early onset Paget disease of bone (PDB) is an unusual disorder. We recently mapped the disorder in a large family from central Illinois with proximal limb girdle muscular dystrophy and PDB to a unique locus on chromosome 9p23.3-q12 (Kovach et al., 2000). We have identified a new 10-member family with dominant PDB and muscular dystrophy. The father, age 70 y., had onset of myopathy at age 41 y. Progression of the disorder began with foot drop in addition to PDB in the L knee. Presently, he has a severe, generalized, distal>proximal weakness and severe PDB causing deformity of his extremities. He is bedridden and requires a tracheostomy and gastrostomy. EMG and muscle biopsy are compatible with a primary dystrophy. His creatine phosphokinase (CPK) levels are currently 40 U/L (normal range 52-336 U/L), however, these levels have been elevated in the past to 776 U/L. Additionally, alkaline phosphatase levels (530 U/L, normal range 98-250) and osteocalcin levels (140 U/L, normal range 2-10) are markedly elevated.

Of his 8 children, a 41 y. old son has a similar muscular dystrophy with onset at age 37 y. His CPK level is 662 U/L. Two daughters, age 43 y. and 40 y, developed PDB at mean age of 39y. with distribution in the pelvis, arms, spine, and tibia. All of the affected children have elevated alkaline phosphatase levels at a mean of 282 U/L.

Haplotype analysis of nine members (4 affected, 4 unaffected, 1 spouse) with a high-density of markers excluded the critical region on chromosome 9p23.3-q12, thus providing evidence for genetic heterogeneity among families with autosomal dominant PDB and muscular dystrophy.

***MECP2* analysis in possible familial Rett syndrome.** H.K. Gill¹, J.P. Cheadle¹, J. Maynard¹, N. Fleming¹, A. Kerr², H. Leonard³, E.M. Thompson³, F. Hanefeld⁴, O. Skjeldal⁵, J. Sampson¹, A. Clarke¹. 1) Institute of Medical Genetics, UWCM, Cardiff, Wales, United Kingdom; 2) University of Glasgow, Department of Psychological Medicine, Gartnavel Royal Hospital, 1055 Great Western Rd, Glasgow. G12 OXH. UK; 3) TVW Telethon Institute for Child Health Research, West Perth, Australia, WA6872; 4) Abteilung Kinderheilkunde, Scheuerpunkt, Neuropaeditre, Georg-August-Universitat, Gottingen, Germany; 5) Department of Paediatrics, Nordland Central hospital, N-8017 Bodo, Norway.

Mutations in the methyl cytosine binding protein 2 gene (*MECP2*) have recently been identified as causative in Rett syndrome. The majority of cases of Rett syndrome are sporadic. However, a number of cases have been thought to be familial because of the occurrence of two affected individuals in the same pedigree. We have undertaken mutation analysis of *MECP2* in 20 affected individuals from 10 families, in whom the possibility of familial Rett has been raised. We identified pathogenic *MECP2* mutations in 6 individuals from 5 families. In 1 family we detected the same *MECP2* mutation in two affected sisters and their unaffected mother. In 4 families, one affected individual was found to have a *MECP2* mutation, but the relative in whom a diagnosis of Rett was suspected, did not carry this or any other mutation. In the remaining 5 families no *MECP2* mutation was detected in any individual. These results suggest that familial Rett syndrome is rare and may be overdiagnosed.

Molecular Detection and Prenatal Diagnosis of Spinal Muscular Atrophy in Iran. *S. Teimourian*^{1,2}, *Y. Shafegati*³, *R. Karimi-Nejad*², *Gh. Baba-Mohammady*², *N. Almadany*², *M. Azad*², *H. Najmabadi*^{2,3}. 1) Biophysics and Biochemistry, Tehran University, Tehran, Ira, Tehran, Tehran, Iran; 2) Karimi-Nejad Pathology and Genetics Center, Tehran, Iran; 3) Welfare and Rehabilitation University, Tehran, Iran.

Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder resulting in the loss of motor neurons in the spinal cord. SMA has an estimated incidence of 1/10,000 with a carrier frequency of 1/60 to 1/40. The survival motor neuron (SMN) transcript is encoded by two genes, SMNT and SMNC. All three forms of SMA that maps to the 5q12 are caused by mutations in the SMNT gene. SMNT exon 7&8 are not detected in ~95% of SMA cases due to either deletion or sequence conversion events. SMNC and SMNT genes are distinguishable only by single base changes in exon 7 and exon 8. We have employed method by (Chang et al., 1997) to compare the telomeric with centromeric portion (T/C ratio) of SMN gene after restriction enzyme digestion followed by acrylamide gel and samples were analysed by silver staining. Out of twelve families we have analysed, nine showed deletions in exon 7 which include 5 affected children with type one phenotype and 2 affected children with type II phenotype. one family with an affected child with SMA showed deletion in exon 8 and only two showed no deletion in exon 7 or 8. Prenatal diagnosis was performed for two out of twelve of these families where trait fetuses were detected.

Abnormal early infantile development in Rett syndrome: expanding the criteria for *MECP2* testing. L. Celle¹, C. Venditti¹, L. Brown², S. Levy³, J. Coplan³, E.H. Zackai¹. 1) Division of Human Genetics and Molecular Biology; 2) Department of Neurology; 3) Division of Child Development and Rehabilitation, The Children's Hospital of Philadelphia, Philadelphia, PA.

Availability of the molecular diagnostic test for Rett syndrome (RS) permits an opportunity to reevaluate current concepts of the natural history of the disorder. Clinical diagnostic criteria developed by the RS Diagnostic Criteria Work Group (Trevathan and Moser, 1988) include presence of normal development in the first 6 to 18 months of life. Several case reports have disputed that finding. Almost half of the surveyed parents reported unusual development and behavior in the first 6 months of life in a study by Leonard & Bower (1998). We report 3 girls with RS confirmed with *MECP2* mutations who initially presented with developmental delay in the first months of life. All were born at full term with normal growth parameters; all showed appropriate social development but had motor delay noted before 6 months. Patient 1 (T158M mutation) first rolled over at 7 mo, sat at 10-11 mo, crawled at 18 mo, and walked at 4 years. Decreased head growth was noted at 16 mo. At 4 years she can no longer crawl, shows dramatic hand apraxia, and gait ataxia. There has been a single seizure episode. Patient 2 (R306C mutation) came to attention at 9 mo due to decreased head growth velocity. She rolled at 5-6 mo, sat at 9 mo. Regression started at 9-12 mo with decreased babbling. Bruxism began at 15 mo, purposeful hand movements were lost at 20 mo and hand wringing was noted at 22 mo. At 24 mo she has no words and has not learned to walk. Patient 3 (1161del6 and 1180del26) first rolled over at 10 mo and walked at 19 mo. Regression was suspected at 9 mo with decreased eye contact and she was diagnosed with an autistic spectrum disorder at 19 mo. Breath holding and gait ataxia begun at 22 mo and hand wringing started at 23 mo. These 3 cases document delayed motor milestones by 6 months of life. We suggest that the diagnosis of RS must be considered in girls with early developmental delay even when they present for evaluations prior to emergence of hand wringing, hyperventilation and regression.

Duplication of 17p11.2: An entity resulting from the homologous recombination of the Smith-Magenis deletion region. *M.A. Deardorff¹, L. Celle¹, R. Finkel², S. Friedman³, N.B. Spinner¹, E.H. Zackai¹*. 1) Division of Human Genetics and Molecular Biology; 2) Division of Neurology; 3) Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA.

Duplication of chromosome 17p11.2, the region deleted in the Smith-Magenis syndrome (SMS) has recently been reported as a distinct entity. Findings in 17p11.2 duplication are predominantly limited to mild to moderate mental retardation and sometimes involve short stature, dental abnormalities, seizures, and behavioral problems. Here we report two additional cases with a duplication of the SMS deletion-specific fragment. Case 1 is a 1-year-old male who was noted to have difficulty feeding, failure to thrive, microcephaly, and developmental delays. At 10 months he was not rolling over or sitting. His mental skills were at 5-7 month level, motor skills at 5 month level, and play skills at 6-7 month level. His dysmorphia was limited to epicanthal folds and downslanting palpebral fissures. Case 2 is a 8-month-old boy who presented with failure to thrive and hypotonia. He acquired head control at 6 months and had not rolled over at 8 months. He had a mildly narrowed cranium, two hyperpigmented spots, and marked hypotonia. High resolution karyotype in both cases revealed cytogenetically visible duplication of 17p11.2. FISH analysis indicated duplication of the SMS fragment (FLIx3, ZNFx3, 86E11x3) and no duplication of the CMT1A probe (PMP22x2). These two cases provide further evidence of focal duplication limited to 17p11.2 (SMS region) resulting primarily in developmental delay with minimal dysmorphia which is similar to the 2 patients reported by Brown et al (1996) and the 7 patients reported by Potocki et al (2000). 17p11.2 duplication is among an increasing number of entities resulting from homologous recombination due to repetitive flanking sequence clusters. High resolution karyotype should be performed when evaluating a patient with developmental delay/MR and minimal dysmorphia and 17p11.2 duplication should be included in the differential diagnosis.

Polymastia In The Genetics Clinic. *J.D. Ranells, B.G. Kousseff.* Division of Medical Genetics, USF, Tampa, FL, 33606.

Polymastia presents a spectrum from normally shaped breast tissue with nipple lacking areola, glandular tissue with areola without nipple, and ectopic breast tissue with neither areola nor nipple.

In our patient database there are 36,665 families evaluated between January 2, 1982 and December 31, 1999 including three patients with polymastia or ectopic nipples.

WS was a 15 year old Caucasian girl with kyphoscoliosis, short stature, SN hearing loss and trainable mental retardation. She was born to a 19 year old 46, XX primigravida. BWT was 2650 g. Microcephaly was present. There was camptodactyly, exotropia, mild pectus excavatum and polythelia along the Hughes lines. By age 16 years, bilateral polymastia developed with glandular tissue, areolas and nipples. The karyotype showed deletion 9q (q31@qter).

LH, a Caucasian 46, XX girl was born with atypical left split hand, absent pectoralis minor and left polythelia in the axilla. There were also left renal agenesis and scoliosis. BWT was 2030 g, length 46 cm, and OFC 32 cm. At age 6 years, LH was nonverbal and nonambulatory. The diagnosis for the limb abnormality was Poland sequence but the overall phenotype extended beyond that of the sequence.

EJ, a 27 month old Caucasian male showed mild pervasive developmental disorder. He was the product of the second pregnancy for the 28 year old para 1-0-0-1 mother who had polymastia with a third breast with glandular tissue, areola and nipple. BWT was 4450 g, length 56 cm and OFC 39.5 cm, megalencephaly; father had that, and bilateral polythelia category 6 according to Kajava's classifications (Duodecim 31:143-170, 1915).

In the USF Genetics Clinics, the experience with polymastia/ectopic polythelia indicated that chromosome abnormalities, syndromic multiple congenital anomalies and atavism are causes of polymastia.

Holoprosencephaly in a fetus with Smith-Lemli-Opitz syndrome (SLOS). *S.A. Farrell¹, W.S. Sirkin², M.J.M.*

Nowaczyk³. 1) Dept Lab Medicine, The Credit Valley Hosp, Mississauga, ON., Canada; 2) Dept Lab. Medicine, North York General Hosp, North York, ON, Canada; 3) Dept Pathology & Molecular Medicine, McMaster University, Hamilton, ON, Canada.

We report on a fetus with SLOS with a median cleft lip, oral frenulum, polydactyly with 2-3 toe syndactyly and alobar holoprosencephaly. Ultrasound showed a fetus with a median cleft, nuchal thickening (6 mm-upper limit 5mm), a 2-vessel umbilical cord, and significant growth lag. At 19 5/7 weeks, the biparietal diameter (BPD) was 18 1/7 weeks and femur length (FL) was 18 6/7. Amniocentesis showed a normal male karyotype. At 23 weeks, BPD was 18 5/7 weeks and FL was 20 5/7. A single cerebral ventricle, bilateral club feet and a small right cardiac ventricle were seen. The pregnancy was ended at 24 weeks. Weight was 483 gm (50th centile for 22 weeks; head circumference was 18 cm (50th centile 21 weeks). There was microcephaly and hypotelorism. The tongue was wedged into a median cleft palate and cleft lip. There was a right frenulum between the tongue and mouth. Both nipples were absent and the nuchal skin was thick. Shoulders were square. Digits were tapered with bilateral postaxial polydactyly. The feet showed bilateral talipes equinovarus, with proximally placed broad great toes. There were 6 toes on the right and syndactyly of toes 2-3 on the left. At autopsy, alobar holoprosencephaly was confirmed, with a posterior ventricular cyst. There was a double inlet left ventricle with a VSD, hypoplastic right ventricle, pulmonary artery stenosis and atrioventricular septal defect. Lungs were unilobar. The urethra communicated with a vagina-like blind-ended pouch. There was a decreased number of Leydig cells in the testes. Amniotic fluid, refrigerated for 3 weeks, was used to measure 7-dehydrocholesterol(7-DHC). The level was 423 umol/L, confirming the diagnosis of SLOS. The ultrasound aspects of this case are unusual with holoprosencephaly, extreme microcephaly, remarkable IUGR at an early gestation, and median clefting with evidence of no tongue movement. This case also illustrates the stability of 7-DHC in refrigerated amniotic fluid and the diagnostic capacity of such a stored sample.

Pervasive Developmental Disorder and 47,XYY males. *K.J. Briggs¹, J. Varsamis², A. Slusky², A. Dawson¹, C. Prasad¹.* 1) Section of Genetics and Metabolism, Health Sciences Centre, Winnipeg; 2) Section of Psychiatry, Health Sciences Centre, Winnipeg.

The association between chromosomal abnormalities and mental retardation is well known. Less well documented is the impact of aneuploidy on childhood Pervasive Developmental Disorder (PDD). XYY syndrome has rarely been associated with Pervasive Developmental Disorder (1 out of 92 children with PDD had XYY syndrome) (Weidmer et al. 1998). XYY syndrome is characterized by accelerated growth and minor dysmorphic features including a prominent glabella (Parker 1969). Full-scale IQ in these patients is usually within normal limits but often lower than siblings, in particular verbal IQ. Language delay is often described in these patients, as is difficulty with social interaction. XYY males tend to be withdrawn and to have difficulty establishing friendships. An association between XYY syndrome and violent behaviour has been postulated but was not supported by prospective longitudinal studies. We report on a fifteen year old boy who was referred for evaluation of developmental delay and behavioural difficulties. He was found to be withdrawn and oppositional, and to have perseveration and concrete thinking. Developmental assessment showed delay in language acquisition. A maternal uncle was withdrawn and dependent, and a second maternal uncle was described as a loner. The patient was in a modified Grade 10 program. He had spent several months in an adolescent treatment centre for behavioural issues, but at the time he was evaluated in Genetics he was living at home. On examination the patient's weight was at the 75th%, height was at the 90th% and he was normocephalic. The patient was non-dysmorphic and physical examination was unremarkable. Performance IQ was in the mildly mentally retarded range. His karyotype was 47,XYY. Psychiatric assessment was of PDD-NOS. It is important to consider aneuploidy in the differential diagnosis of patients presenting with PDD.

Longitudinal personality changes as measured by the MMPI in presymptomatic HD gene carriers. S.C.

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A sample of individuals at-risk for Huntington Disease (HD) and reporting themselves as asymptomatic participated in a longitudinal case-control double blind study to determine whether longitudinal changes in personality as measured by the MMPI can be detected among clinically presymptomatic individuals carrying the expanded Huntington disease (HD) allele. The participants, examined an average of 3.7 years apart, were categorized into one of two groups: (1) presymptomatic gene carriers (PSGC) (n=12), defined as those individuals with an expanded HD gene (³ 38 CAG repeats) but who did not have sufficient neurologic symptoms to warrant a clinical diagnosis of manifest HD; and (2) nongene carriers (NGC) (n=31), defined as those individuals with 2 unexpanded HD alleles (< 32 CAG repeats). In addition to molecular testing to determine the number of CAG repeats in the HD gene, the clinical assessment included a quantified neurological examination and an abbreviated Minnesota Multiphasic Personality Inventory (MMPI). The abbreviated MMPI included the Hypochondriasis, Depression, Psychasthenia, neuroticism, hostility-cynicism, and irritability scales and the Harris Subscales of Depression: Subjective Depression, Brooding, and Psychomotor Retardation. The results on the MMPI were evaluated for differences between NGC and PSGC using a one-factor between subjects ANOVA. For the PSGC, a greater increase in abnormality over time was observed for the MMPI scales, hostility-cynicism (p=0.04) and irritability (p=0.005), when compared with the NGC. Among both the PSGC and NGC, no significant correlation was found between the number of CAG repeats and the change in MMPI score from the original to the return visit. This study provides significant evidence for increasing irritability and hostility-cynicism in presymptomatic gene carriers prior to the onset of overt clinical symptoms.

Trisomy 14q detected by telomere FISH: Unraveling parent-specific effects of 14q dosage. *V.R. Sutton, S. Lalani, C.D. Kashork, L.G. Shaffer.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

We present the first case report and molecular cytogenetic studies of trisomy 14q detected by telomere FISH. Our patient is a 2 year old girl with growth and developmental delay, myelomeningocele, partial agenesis of the corpus callosum, hypertelorism, tented mouth, simple ears, small mandible and congenital heart disease (atrial and ventricular septal defects with subaortic conus). G-banded chromosome analysis was normal. A set of FISH-based, telomere region-specific probes revealed trisomy for 14q: ish der(22)t(14;22)(qter;p11.2)(14qtel+). Parental FISH studies established that the mother is a balanced carrier for this translocation: ish t(14;22)(qter;p11.2)(14qtel-;14qtel+). Chromosome 14 is known to have regions of imprinting and distal 14q has been suggested to contain imprinted genes. Because our patients features are not similar to maternal or paternal uniparental disomy (UPD) case reports, we suggest that either the area defined by the telomere probe does not contain imprinted genes, or that the phenotype found in UPD 14 is produced by deficiency of a particular parental allele(s), rather than a double dose of a particular parental allele. This case of trisomy 14q illustrates the great utility of telomere-specific FISH probes in finding cryptic rearrangements and aids in uncovering the effects of parent-specific expression for regions of 14q.

Clinical features of male with short stature, dextrocardia and retinal retraction syndrome with XY translocation suggest possible existence of a new Xpter gene locus involved in situs determination. *P. Bitoun*¹, *B. Benzacken*², *J. Gaudelus*³. 1) Dept Gen Med, CHU Paris-Nord, Hosp Jean Verdier, Bondy, Cedex, France; 2) Dept Embryo-Cytogenetics; 3) Dept Pediatrics.

We report on an 9 year old male seen for genetic counseling of a malformation syndrome with short stature , dextrocardia and right retinal retraction syndrome. Short stature was diagnosed at age 4 and treated by age 7 for borderline GH deficiency. Brain MRI scan showed normal pituitary gland as well as brain structures and maturation. Dextrocardia discovered at age 1 seems isolated with normal situs of liver and lungs on chest X and lung x rays. Right sided low vision was diagnosed at age 3 and angiography showed an epiretinal membrane. Progressive traction syndrome of retina developed causing visual loss requiring multiple surgeries. This young boy has normal intelligence and development. He has minimal dysmorphism with a short upturned nose and low-set ears, no missing incisors or cleft and normal smell sensation. His peripheral blood high resolution caryotype initially showed a 45 X caryotype with a mosaic with 1 or 2 minute ring chromosomes. Using FISH the SRY probe showed a Y translocated on the Xpter region. Centromeric X probes confirmed the minute ring chromosomes were X chromosomes. Caryotype showed a mosaic variegated aneuploidy with 58% cells with 45 tY;X (p?,pter); 38% cells with 45 tY;X (p?,pter), + X mar; 4% cells with 45 tY;X (p?,pter), + X mar, + X mar. The short stature possibly results from SHOX gene haplo-insufficiency (located at Xp22.32) but it is still unclear which genes might explain the dextrocardia (the only known gene on the X involving right-left pattern determination is ZIC3 at Xq26) or the retinal retraction syndrome. Mother has normal caryotype, Paternal caryotyping and Investigation of the breakpoints on the X and the Y may help explain the pathology observed in this patient. The patient's features suggest there may be another gene involved in situs determination in the Xpter region.

Genetic Etiology of Blindness. A prospective study at a tertiary Pediatric Hospital. *A.D. Iglesias¹, M. Torrado², C. Barreiro²*. 1) Pediatrics, Jacobi Medical Center, New York, NY; 2) Hospital de Pediatria, Juan P. Garrahan, Buenos Aires, Argentina.

During one-year, 30 patients with blindness of presumptive genetic etiology were studied at the Department of Genetics. Patients were screened at Ophthalmology. Inclusion criteria included the WHO definition of blindness. Exclusion criteria included environmental causes of blindness, retinoblastoma, and ambliopia. Initial diagnosis included cataracts, leucocoria, microphthalmia, glaucoma, anophthalmia, lens subluxation, aniridia, nystagmus, myopia, and strabismus. Ages range from 19 days to 13 years. Males represented 2/3 of the cases. After genetic evaluation, 10 cases (33%) showed mental retardation (MR) including Down, Lowe, and Lenz syndromes, and COFS disease. Eighteen (60%) cases presented one or more associated defects including MR (8), facial dysmorphic features (7), microcephaly (2), hand anomalies (2), failure to thrive, axial hypotonia, marfanoid habitus, short stature, and phenotype of Down's. Definitive diagnosis of a genetic entity was made in 9 cases including Down, Lenz, COFS, Goldenhar, Rieger, Lowe, Stickler, and Weil-Marchesani syndrome (2) and COATS disease. In 22 cases (73.4%) a defined genetic disorder was found. Seventeen cases were mendelian including 5 autosomal dominant (AD), 5 autosomal recessive (AR), and 2 X-linked recessive (XLR). One case was chromosomal, and 5 cases were multifactorial. Single ophthalmologic entities with genetic inheritance were also identified. Seven were cataracts (1 AD, 3 AR, 3 XLR), 2 glaucoma (1 AD, 1 XLR), 2 XLR coloboma, 1 AD anophthalmia, 6 cases of anophthalmia (2 AD, 2 AR, 2 XLR), and 2 cases of retinitis pigmentosa (1 AD, 1 AR). Overall, 70% of the cases had a definitive diagnosis. Hereditary patterns of inheritance as well as the natural history, prognosis, therapy and genetic counseling for the patients and their families were discussed. The opportune, accurate and timely referral of patients with blindness of presumptive genetic etiology were the clues for a definitive diagnosis. Future delineation of complex entities, as well as single defects of genetic etiology, will help to understand the genetic basis of childhood blindness.

Are specific eye findings a useful predictor of the presence of associated major anomalies in anophthalmia/microphthalmia? *A.S. Schneider, T.M. Bardakjian, A.L. Aronson, M.A. Dwyer.* Dept Pediatrics, Albert Einstein Medical Ctr, Philadelphia, PA.

The Anophthalmia/Microphthalmia(A/M)registry was established in 1993 to assemble a national database of individuals with A/M, to determine the local and national incidence of A/M and to undertake a descriptive analysis of the registry population. Analyzing our collection of clinical data on 131 cases of unilateral and bilateral anophthalmia or microphthalmia, we have tried to determine whether specific eye findings can be used to predict the likelihood of other anomalies in the individual. The total number of cases reviewed is 131; 68 males and 63 females. Of the females, 28 have unilateral anophthalmia or microphthalmia (15 left, 13 right) and 35 have bilateral A/M. 3/13 (23%) of females with left microphthalmia had other significant findings with brain and cardiac anomalies. The 2 females with left anophthalmia had no other anomalies. One of the 4 females with right sided anophthalmia had hemifacial microsomia. 3/9 (33%) of females with right microphthalmia had associated brain abnormalities. The total number of males with unilateral microphthalmia is 24 (13 left, 11 right). Only 1 male with left unilateral microphthalmia had associated anomalies. 4/11(36.6%) males with right microphthalmia had other anomalies. There are only 2 males with unilateral anophthalmia (8% of our sample), both have anomalies with syndromal diagnoses. Males and females with bilateral A/M present with a wide range of findings. 51% of males and 63% of females have significant associated findings including cardiac, renal and brain anomalies Our analysis has demonstrated that bilateral A/M in both males and females as well as unilateral A/M in females are associated with a significant number of anomalies of other systems. We therefore recommend that all individuals with A/M be evaluated by a Clinical Geneticist and advocate a full work up for most of these patients. However, males with unilateral A/M in this sample, even with coloboma or PHPV, have a low rate of associated anomalies and the workup may be limited based on clinical assessment of the examining Geneticist.

PAX2 mutations and Renal-Coloboma syndrome. L.A. Schimmenti¹, A.C. Gelb¹, S. Militar¹, P.A. Sieving², J.A. Kemp², G.W. Chung³, A.O. Edwards³, Y.H. Zhang¹, G.S. Manligas¹. 1) Departments of Human Genetics and Pediatrics, UCLA, Los Angeles, CA; 2) Ophthalmology & Visual Science, University of Michigan, MI; 3) Department of Ophthalmology, UT Southwestern Medical Center, TX.

We report two new mutations identified in the coding region of *PAX2*. Renal-Coloboma Syndrome, an autosomal dominant disorder associated with mutations in *PAX2*, is characterized by colobomatous eye defects, renal hypoplasia, vesicoureteral reflux, high frequency hearing loss and rarely central nervous system abnormalities. In our search to define phenotypes associated with *PAX2* mutations, we identified a three-generation family and an unrelated isolated proband with Renal-Coloboma Syndrome. In the three-generation family, the proband was an 8 month old boy with nystagmus and bilateral optic nerve dysplasia. His acuity at age 6 is 20/80 OD; 20/600 OS, and he currently has normal renal function. His mother and maternal grandmother also have bilateral optic nerve dysplasia and high tone hearing deficiency; both had renal disease leading to renal transplants. SSCP analysis of the *PAX2* coding region, followed by direct sequencing of exon 3, revealed a novel nonsense mutation, Arg114X (C886>T), in one allele of all three. The unrelated isolated proband is a 9 year old girl with congenital renal hypoplasia who developed endstage renal disease and was transplanted at age 7. She enjoys normal vision (20/20 OU). Optic nerve colobomas were discovered incidentally when the patient was treated for CMV retinitis. Mutation analysis revealed a single base pair deletion in exon 2, delT602 with a resultant frameshift and stopcodon (UGA) 8 codons downstream. As in the majority of patients with *PAX2* mutations, these patients have mutations in the exons encoding the paired domain. Of importance, systemic involvement in both probands was identified surreptitiously and suggests that a history of renal disease should be sought in individuals with colobomas and that examination for colobomas should be entertained in individuals with congenital renal hypoplasia. Identification of mutations in *PAX2* in such individuals will have genetic counseling implications.

Clinical Features of Four Males and an Obligate Carrier in a Family with Lenz Syndrome. *N.M. Reynolds¹, S. Forrester¹, M.J. Kovach¹, R. Urban², V.E. Kimonis¹*. 1) Dept of Pediatrics, Southern Illinois University School of Medicine, Springfield, IL; 2) Dept of Ophthalmology, Tampa General Hospital, Tampa, FL.

Lenz syndrome is a rare X-linked recessive syndrome first described by Lenz in 1955. Clinical features include anophthalmia, microcephaly, mental retardation, cardiac, skeletal, urogenital, external ear, and digital, anomalies. We present three brothers (ages 15 years, 9 years, and 18 months) and a maternal uncle (age 27 years) with congenital anophthalmia, hypotonia and moderate to severe mental retardation. Dysmorphic features include dysplastic ears, high arched palate, pectus excavatum, finger and toe syndactyly, clinodactyly and fetal pads. Other features include scoliosis, cardiac and renal abnormalities. Obligate carriers have a history of recurrent spontaneous losses. One carrier has dysplastic ears and syndactyly of the 2-3rd toes bilaterally; features which may be helpful in identification. Fourteen previously reported cases were additionally reviewed. Mental retardation was present in 100%, 93% had growth retardation, 69% had microcephaly, 88% had ear anomalies, 76% had dental anomalies, 12% had a congenital heart defect, 50% had a urogenital anomaly, 47% had a spinal deformity and 69% had anomalies of the fingers or toes.

Linkage and haplotype analysis in this family indicates that the gene is located in a 17.65 cM region on chromosome Xq27-Xq28 flanked by microsatellite markers DXS1232 and DXS8043. This region overlaps the anophthalmos locus ANOP1, but excludes the OCRL locus for Lowe oculocerebrorenal syndrome. Candidate genes involved in neuronal development that map to this critical interval include CXORF1 and KIAA0006. This is the first report of linkage analysis in Lenz microphthalmia a unique disorder associated with mental retardation and multiple anomalies.

X-linked omphalocele with obstructive uropathy. *R.M. Ruzicka, E.R. Wassman, J. Siegel-Bartelt.* Perinatal Alliance Medical Group, Pasadena, CA.

A 23-year old G3 P2 TAB1 Hispanic female patient presented for preconceptional genetic counseling with a striking family history of X-linked omphalocele. Our patient had three male pregnancies, two of which died in the newborn period with omphalocele in Mexico and one of which was terminated at 15 weeks gestation due to the detection of omphalocele by ultrasound. Our patients first son was also noted to have hypertelorism, a congenital heart defect, low-set ears, and posterior urethral valves. The second son was noted to have omphalocele, obstructive uropathy leading to oligohydramnios, and a two-vessel umbilical cord. Amniocentesis performed during the second pregnancy yielded a normal male karyotype. The family history was also significant for two brothers, one nephew, one maternal uncle, and one maternal male cousin of our patient who died in the newborn period with omphalocele. Detailed information on other possible anomalies of the other affected family members is not available. The pedigree is highly suggestive of an X-linked recessive omphalocele condition, similar to that reported previously by Havalad et al (1979). The specific combination of obstructive uropathy with omphalocele in the family we report is uncommon. The differential diagnosis of X-linked syndromes with omphalocele includes Melnick-Needles syndrome, otopalatodigital syndrome type II, thoracoabdominal syndrome, focal dermal hypoplasia, and Opitz/BBB syndrome. However, none of these conditions fits the spectrum of anomalies described in this family particularly well. The patient was counseled that unless a particular mutation or diagnosis can be identified in this family, the options for prenatal diagnosis in a future pregnancy are limited to ultrasound and sex determination through amniocentesis, CVS, or preimplantation genetic diagnosis. The anomalies seen in this family may represent a new X-linked omphalocele syndrome or a new variation of a previously described syndrome.

Familial prader willi syndrome with unusual findings. *L.A. Bastaki, S.A. AL-AWADI, S.J. ABULHASAN, F.M. MOHAMMED, S.A. GOUDA, K.K. NAGUIB.* Maternity Hosp, Kuwait Medical Genetic Ctr, Khaldiya, kuwait, Kuwait.

Herein we are reporting 6 sibs with Prader Willi(PWS)(5males and one female).All of them show the cardinal features of PWS except one who does not fulfil the required criteria.chromosomal study confirmed normal karyotype but FISH technique using specific DNA probes revealed del(15)(q11&12) trasmitted from the mother. chromosomal study and FISH technique of the father were normal.Three unusual findings were reported;the multiplicity of the affected sibs;the unusual clinical findings in one of the sibs and the maternal origin of the deleted chromosome 15.The possible explanations are discussed.

Results of clinical and molecular evaluation of 14 hereditary ataxia families: Mysteries Remain. *M.E. Ahearn¹, N. Potter², H. Zoghbi³, J. Mater¹, W. Bradley¹, L. Baumbach¹.* 1) Univ Miami School of Medicine, Miami, FL; 2) Univ Tennessee Med Ctr, Knoxville, TN; 3) Baylor College of Medicine, Houston, TX.

The hereditary cerebellar ataxias represent a clinically and genetically heterogeneous collection of progressive neurological disorders. A number of causative genes have been identified but new genes causing specific forms of ataxia are constantly being discovered. Here we report our clinical and molecular investigations of 14 families with a pure or mixed cerebellar ataxia. Of these families, three with significant family history remain undiagnosed as to their genetic etiology. Trinucleotide repeat expansions were found as follows: 1 *SCA1*, 2 *SCA2*, 2 *SCA3* and 1 *SCA6* (with two samples pending completion). Five individuals (three with significant family history) have tested normal for all the known ataxia genes including the recently described *SCA8*. The three families with significant history are the subject of further investigation. Family 1 is a large family with seven affected members spanning three generations. This family exhibits a late onset cerebellar ataxia with dysarthria, lower extremity and truncal ataxia. Family 2 has four affected individuals spanning three generations. Clinical symptoms present in the family include balance and gait difficulties, dysarthria, nystagmus and peripheral sensory nerve problems and also shows anticipation with regard to age of onset. Family 3 who represent an autosomal recessive pattern of inheritance, has four affected sisters, with clinical symptoms very similar to Friedreich's ataxia.

In summary, this study indicates that although the genetic basis for many ataxia patients can be determined in families with strong histories, additional genes that cause ataxia remain to be found.

Thalassaemia intermedia in Iran: Molecular determinants. *M.T. Akbari*¹, *E. Dudman*², *M. Izadyar*³, *M. Kleanthous*⁴, *M. Petrow*⁵. 1) Dept Genetics, Sch Medicine, Tehran Univ Medical Sci, Tehran, Iran; 2) Perinatal Centre, Dept. of Obstetrics and Gynecologist, University college of London, London UK; 3) Dept. Pediatrics, School of Medicine, Tehran University of Medical Sciences, Tehran Iran; 4) CING, Nicosia, Cyprus; 5) Perinatal Centre, Dept. Obstetrics & Gynecology, University College London, London UK.

We report the result of investigation on the molecular foundation of thalassaemia intermedia in 100 Iranian patients. These patients were clinically heterogeneous. The age at diagnosis varied considerably amongst them with mean age of over 7 years. They developed very few symptoms and just a few number of them needed occasional blood transfusion. Other therapeutic interventions were splenectomy in a third of them and administration of folic acid and hydroxy urea in some patients. Hb concentration in the majority of the patients was between 7-10 g/dL and in a quarter of them it was between 10-12 g/dL. Over 90% had high HbF levels. This indicates a substantial elevation of expression of the gamma globin genes. All patients were examined for the -158 C--->T substitution in the G-gamma promoter locus, recognized by cutting with the restriction enzyme XmnI, and over three quarters of the cases had the mutation in either homozygous or heterozygous form. The patients were also scanned for beta-globin gene mutations. Over 60% of the patients had the IVSII-1 mutation (the most common in Iranian populations) in either homozygous or heterozygous combinations. Almost all the patients with IVSII-1 mutation had the XmnI marker concurrently. Examining the patients for both molecular determinants, it was established that there is physical linkage between the XmnI marker and the IVSII-1 allele in the cases who carried the two mutations. It is a known fact that the coexistence of the XmnI marker and IVSII-1 allele in cis situation under the condition of hematopoietic stress usually contributes to the overproduction of HbF causing HPFH. It seems this phenomenon endows mild features of intermedia in a big number of the Iranian thalassaemia intermedia patients.

Siblings with cutis laxa type II(Debre type). *M.T. Greally*¹, *W. Agab*², *A.H. Ebrahim*³, *K. Ardatt*⁴, *P.H. Byers*⁵. 1) Col Medicine & Med Sci, Arabian Gulf Univ, Manama, Bahrain; 2) Pediatric Dept, BDF Hospital, Bahrain; 3) Pediatric Dept, Salmaniya Medical Complex, Manama, Bahrain; 4) Pediatric Clinic, Toobli, Bahrain; 5) Dept Pathology, Univ Washington, Health Sci Bldg, RM E511, Seattle WA, USA.

Cutis laxa type II (Debre type), an autosomal recessive form of cutis laxa, is a disorder of elastic fibers characterized by joint laxity, large fontanelle and developmental delay. Additional features include distinctive facial dysmorphism, an Ehlers-Danlos-like healing defect, hair abnormalities and central nervous system abnormalities. We present siblings, a brother and sister of consanguineous Arab parents, with features of this syndrome. The boy is more severely affected than his sister with the additional clinical findings of abdominal herniae, bilateral renal stones and multiple episodes of severe joint hematomata following minor trauma. Investigations included type I and type III collagen synthesis and secretion, both of which were normal. Skin electromicroscopy (EM) studies showed apparently normal collagen fibrils while the elastic fibers were small and had a 'moth-eaten' appearance. These findings were not as striking as those seen in other cases of cutis laxa type II where extreme rarefaction of elastic fibers has been described. The variety of clinical findings in this disorder and the reported differences in skin EM studies of elastic fibers, raise the question of genetic heterogeneity in this form of cutis laxa. E-mail address: mgreally@agu.edu.bh.

Facial Clefting Syndrome: A new autosomal recessive entity in Mexican population. *D. Saavedra*¹, *L. Cornejo*², *B. de la Fuente*³, *F. Ortiz-Monasterio*², *M. Muenke*⁴. 1) Craniofacial Genetics Research, Hospital "Dr. Manuel Gea Glz.", Mexico City, Mexico; 2) Medicine School, Health Sciences Institute, Autonomous Hidalgo State University, Pachuca de Soto Hidalgo, Mexico; 3) Medicine Faculty, Autonomous Nuevo Leon University, Monterrey Nuevo Leon, Mexico; 4) Medical Genetics Branch, NHGRI, NIH, Bethesda MD, USA.

Introduction.- Any disruption of the anatomic continuity of the craniofacial structures is considered as a cleft. According to Tessier, the rare craniofacial clefts are numbered from 0 through 14 and they follow constant lines across different structures. Gollop described in 1981 the Fronto-Facio-Nasal Dysplasia (FFND), but regarding the description and the clinical pictures, this patient resemble fissured patients as described by Tessier. **Material and Methods.-** A field study was carried out in South Baja California Mexico, in order to determine the existence of a common factor between two affected siblings with facial clefts born from the same couple. **Results.-** Thirteen patients with the same phenotype consisting on 1, 2, 3, 4, 10, 11, 12, 13, and, 0-14 craniofacial clefts were found consisting by: widened frontal with hair lines leading toward the clefts, hypertelorism, bilateral coloboma of eyebrows, eyelashes and eyelids, hypoplastic alae nasi and cleft lip/palate. All patients are descendents of consanguineous couples, related among them by a common ancestor. This pedigree was demonstrative of an autosomal recessive inheritance pattern (AR). **Conclusion.-** We report a new syndrome consisting on craniofacial clefts inherited as an AR trait and therefore we consider that the patients diagnosed with FFND have this new syndrome.

A common mutation in the methylenetetrahydrofolate reductase gene is not a risk factor for Down syndrome in a population-based study. *M.B. Petersen¹, M. Grigoriadou¹, M. Mikkelsen².* 1) Dept Genet, Inst Child Health, Athens, Greece; 2) Dept Med Genet, JF Kennedy Inst, Glostrup, Denmark.

Trisomy 21 is the most common chromosome abnormality in liveborns and is usually the result of nondisjunction of chromosome 21 in meiosis in either oogenesis or spermatogenesis. Advanced maternal age is the only well documented risk factor for maternal meiotic nondisjunction, but there is still a surprising lack of understanding of the cellular and molecular mechanisms underlying meiotic nondisjunction. It would be of big medical importance to identify younger mothers at increased risk for Down syndrome (DS). A recent study demonstrated increased plasma homocysteine concentration in the mothers of children with DS, partly explained by a higher frequency of the 677C>T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene than in control mothers (Am J Clin Nutr 1999;70:495-501). The risk of having a child with DS was 2.6-fold higher in mothers with the 677C>T substitution in one or both alleles than in mothers without the substitution. The study was based on a small number of mothers (n=57), was not population-based, and the parental origin of the extra chromosome was not determined. We therefore analysed the MTHFR 677C>T mutation in a population-based study of DS in Denmark, where the origin of nondisjunction was determined by DNA microsatellite analysis. The material consisted of 177 mothers of children with DS (non-mosaic free trisomy 21) with origin of nondisjunction in maternal meiosis I or II. The frequency of the mutant allele (677T), which in the homozygous state is associated with mild hyperhomocysteinemia, in the DS mothers was 27.7%, not significantly different from the frequency in the fathers (29.8%) and not significantly different from the frequency in Danish controls (29.0%, n=1,084). Furthermore, there was no difference in the frequency of the 677T allele between mothers with meiosis I errors (27.5%, n=122) and mothers with meiosis II errors (28.2%, n=55). We conclude that the common MTHFR 677C>T mutation is not likely to be a maternal risk factor for DS and that the previously published preliminary findings were probably due to the small sample size.

Patient with Autistic Disorder and 46, XY, dup (7) (p14.1p11.2). *S.L. Donnelly¹, C.M. Wolpert¹, M.L. Cuccaro², C.W. Poole¹, H.H. Wright², J.R. Gilbert¹, M.A. Pericak-Vance¹.* 1) Duke University Medical Center, Durham, NC; 2) W.S. Hall Psychiatric Institute, University of South Carolina, Columbia, SC.

We identified a 25-year-old male with Autistic Disorder (AD) and a history of a chromosome 7 anomaly. The patient has several dysmorphic features including a high forehead, shallow orbits, a large bulbous nose, macrognathia, brachydactyly, mild scoliosis, and feet that measure at the third percentile. Previous metabolic screens and neurological exams were within normal limits. The Autism Diagnostic Interview Revised (ADI-R) was conducted to further characterize the patient. He has stereotyped speech, odd use of gaze, circumscribed interests and repetitive and ritualistic behaviors. High-resolution chromosome analysis was performed for the patient to confirm the report of a chromosome anomaly. It was revealed that this patient has the following chromosome complement, 46,XY,?dup(7)(p14.1p11.2). An extra segment was observed on the proximal short arm of chromosome 7. The G-band pattern suggested an inverted duplication of 7p11.2-p14.1. In order to confirm the chromosomal origin of this extra segment, chromosome painting was performed. Fluorescence in situ hybridization (FISH) using a whole chromosome 7 DNA probe (Cytocell, Inc.) confirmed that the extra chromosomal material is derived from chromosome 7. Therefore, this patient is partially trisomic for chromosome 7. This type of chromosome anomaly is rare, with about 30 cases having been reported, and is usually associated with a familial balanced translocation. Therefore, high-resolution chromosome analysis of cultured blood lymphocytes was done for both parents of this patient. A normal chromosome complement was found for both parents. It is unclear whether the patient's AD phenotype is causally related to the 7p duplication. However, Rodier et al. (1997) reported that polymorphisms of the HOXA1 gene located on 7p15-p14.2 are over represented in patients with AD. These data suggest that this region of 7p needs to be investigated further as playing an etiologic role in the genetics of AD.

Molecular characterization of a balanced translocation t(X;21)(p11;q22) associated with non-specific mental retardation. *F. Laugier-Anfossi¹, D. Depetris¹, M.G. Mattéi¹, B. Delobel², M.F. Croquette², M. Fontès¹, L. Villard¹.* 1) Inserm U491, Faculté de Médecine La Timone, Marseille, FRANCE; 2) Centre de Génétique Chromosomique, Hôpital Saint-Antoine, Lille, France.

Non-specific X-linked mental retardation (MRX) phenotypes have been described in a large number of families. Linkage analysis performed in these families has allowed to map the disease loci on the human X chromosome. However, only a small number of such families (0.5-1%) have mutations in the six MRX-causing genes that were identified so far. It is thus obvious that numerous as yet unidentified genes reside on the X chromosome and cause an MRX phenotype when mutated. One powerful way to isolate such genes is to take advantage of balanced translocations involving the X chromosome in female patients presenting a non-specific mental retardation phenotype. We have studied such a patient presenting a de novo balanced translocation t(X;21)(p11;q22). She has moderate MR and very slight dysmorphic features. In order to localize the translocation breakpoint on the X chromosome, several YAC clones were used in FISH experiments and one YAC was shown to cross the breakpoint. We subsequently used PAC clones to further narrow the breakpoint region and we have shown that the breakpoint is containing within a 170 kilobases PAC clone in Xp11.2. Fortunately, the PAC clone sequence was available at the Sanger Center and we used this genomic sequence to 1- design subclones in order to further narrow the breakpoint region and 2- use exon prediction programs to identify potentially transcribed regions. Several exons are located inside this PAC clone and experiments are currently underway to determine if a gene is interrupted by the X chromosome breakpoint in the patient.

Agyria/pachygyria is associated with 7q31-qter duplication. *N. Morichon-Delvallez¹, H. Elghezel¹, S. Fontaine¹, C. Esculpavit¹, Y. Martinovic¹, M.C. Aubry², P. Sonigo², A. Benachi², S. Romana¹, M. Vekemans¹, F. Encha-Razavi¹.* 1) Dept Genetics, Hosp Necker-Enfants Malades, Paris, France; 2) Prenatal Diagnosis Center, Hosp Necker-Enfants Malades, Paris, France.

Agyria/pachygyria characterized by absence and/or rarefaction of brain convolutions is a common human brain malformation as shown by CT scan and MR Imaging. So far at least two genes, LIS1 on chromosome 17 and DC on chromosome X have been causally related to this genetically heterogeneous condition. Here we report on a novel association of agyria/pachygyria with a duplication of the long arm of chromosome 7. A 28 years old woman was referred at 34 weeks of pregnancy for counselling because ultrasound examination revealed bilateral ventriculomegaly. MR Imaging detected abnormal gyration and vermis hypoplasia. Cytogenetic study including spectral karyotyping and FISH analysis showed a 7q31-qter duplication due to a de novo (Y;7) translocation. After genetic counselling the parents decided to interrupt the pregnancy. Fetopathological examination was carried out : the fetus, a euthrophic boy presented slight facial dysmorphic features with arachnodactyly. No macroscopic and microscopic abnormalities were found at autopsy. X-Ray evaluation was normal. Neuropathological examination detected a pachygyric brain (weight : 310 g ; NI : 340 g) and a cerebellar vermis hypoplasia (weight : 13 g ; NI : 20 g). Microscopical study of brain sections showed a six layered cortical plate, with sub-ependymal and interstitial heterotopias. Vermian folia had a focal dysplastic pattern with purkinje cells rarefaction. Basal ganglia as well as the commissural and cortico-spinal tracts were well developed. The postnatal phenotype of 7qter duplication is usually associated with slight facial dysmorphism and mental retardation but to our knowledge, its association with agyria/pachygyria has not been described so far. Further studies are needed to confirm the association of 7q31-qter duplication and agyria /pachygyria, and to establish a causal relationship.

Hypoplastic left heart in a female infant with partial trisomy 4q due to de novo 4;21 translocation. *M. Velinov^{1,5}, H. Gu¹, K. Yeboa³, D. Warburton², T. Tubo², S. Dhuper⁴, S. Lanter⁴, D. Delprino⁴, G. Kupchik⁵, E.C. Jenkins^{1,4}.* 1) NYS Institute for Basic Research, Staten Island, NY; 2) Department of Genetics and Development, Columbia University, NY, NY; 3) Department of Clinical Genetics, Columbia University, NY, NY; 4) Brookdale University Hospital Medical Center, Brooklyn, NY; 5) Maimonides Medical Center, Brooklyn, NY.

We present a female infant, who was born at full term, with birth weight 7lbs, 13oz and presented with mild dysmorphic features: sloping forehead, hypertelorism, short and mildly webbed neck, and was subsequently identified to have congenital heart defects: hypoplastic left heart with aortic atresia and hypoplastic aortic arch, VSD and a nonrestrictive atrial communication. The chromosome study showed an unbalanced chromosomal translocation with additional material from 4q translocated onto 21q. This resulted in partial trisomy 4 and minimal monosomy 21. The derivative chromosome was characterized using M-FISH and whole chromosome painting. The karyotype was finally determined as 46,XX,der(21)t(4;21)(q25;q22.3).ish(wcp4+;wcp21+). The parental karyotypes were normal with the exception of mosaicism for a marker chromosome 2 in the father. This paternal marker was considered not to be related to the de novo translocation in the child. Additional analysis with FISH probes specific for 21q11.2, 21q21.2, 21q22.1 and 21q22.3 as well as typing with two microsatellite markers mapping on 21q21 ruled out a possible duplication of 21q on the derived chromosome. The patient underwent stage 1 Norwood procedure to manage her heart defect. Poor feeding and failure to thrive complicated the postsurgical period. At 4.5 month of age the child presented with significant growth delay. Hypoplastic left heart syndrome is a severe congenital defect accounting for at least 10% of the neonatal death due to structural heart defects. This heart abnormality is seen with increased frequency in relatively common chromosomal aberrations, such as monosomy X, trisomies 21, 18, 13. Our report provides for the first time evidence for the possible role of genes located on chromosome 4q, distal to band 4q25 or on the terminal 21q fragment in the pathogenesis of this heterogeneous condition.

Chromosomal capping or interstitial deletion in a case of Wolf-Hirschhorn syndrome. *M.J. Macera, and R.S. Verma.* Division of Molecular Medicine and Genetics, Wyckoff Heights Medical Center, Brooklyn-New York Hospital/Weill Medical College of Cornell University, New York, NY.

The Wolf-Hirschhorn syndrome (WHS), regarded as a contiguous gene syndrome, is characterized by mental and growth retardation, maxillary hypoplasia, midline defects, skeletal abnormalities and a high nasal bridge with large protruding eyes, giving a "Greek Helmet" appearance. This syndrome results from the deletion of the short arm of chromosome 4 with the 165 kb critical region localized to 4p16.3. Varying degrees of expression have been associated with different degrees of deletions. We were referred a boy showing the severest symptoms of WHS. GTG banding of the proband identified a 46,XY,del(4)(p16.1) karyotype in his peripheral blood. Both of his parents were cytogenetically normal. FISH analysis with a probe specific for the Wolf-Hirschhorn region (Vysis) plus the 4 centromere (cep4) probe documented the deletion of the WHS region on his del(4) chromosome. Signals were present on both pairs of his parents' chromosome 4. The 4p telomere probe (4TelVysion Vysis) specific for the unique telomere sequence, gave a positive signal only on the normal chromosome 4 in the proband. No signal was detected over the del(4). We then applied a probe specific for the TTAGGG repeat sequence (Cytocell) that caps the distal ends of each chromosome. Signal was detected over the distal p del(4) chromosome, identifying the presence of the (TTAGGG)_n cap. His karyotype is 46,XY,del(4)(p16.1).ish (LSI WHS-, TelVysion4-, PanTelomere+). These results suggest two possible pathways that describe the deletion: 1) an interstitial deletion with the distal break point located proximal to the (TTAGGG)_n capping sequence and distal to the unique telomere sequence or, 2) more likely, a terminal deletion containing all the material from 4p16.1 to pter including the entire telomere complex, later capped through the action of telomerase, which stabilized or "healed" the chromosome by adding a new (TTAGGG)_n cap to its end. These findings suggest that all terminal deletions should be evaluated by molecular techniques.

Meiotic pairing resulting in an unusual recombinant chromosome involving heterochromatin in a fetus derived from a paternal translocation [t(1;9)(p13;q32)]. V.R. Pulijaal¹, S. Gogineni¹, A. Mohammed¹, H.M.N. Nitowsky¹, S. Gross¹, H. Mootabar², M. Rigs², M.J. Macera³, R.S. Verma³. 1) Dept OB/GYN, Montefiore Medical Center-Albert Einstein College of Medicine, Bronx, N.Y.; 2) Columbia Prenatal Associates of Westchester, Yonkers, N.Y; 3) and Wyckoff Heights Medical Center, Brooklyn New York Hospital/Weill Medical College of Cornell University, New York, N.Y.

Human chromosome 9 is one of the most morphologically deviant chromosomes due to the highly variable heterochromatin of the secondary constriction region [qh]. Morphological variation of the h region in a homologue chromosome 9 do not have aberrant consequences during meiotic pairing and these variants have been inherited by the progenies without clinical consequences. A 35 year old women [G1P0] was referred for genetic amniocentesis. Cytogenetic findings with the GTG-technique revealed an abnormal 46,XY,t(1;9)(p13;q33),rec(9)dup(q11q13)phqh karyotype from her amniocytes. Parental chromosomes revealed that the mother was apparently normal while the father has a balanced translocation between chromosomes 1 and 9 in addition, the other chromosome 9 has a complete pericentric inversion involving the qh region i.e. 46,XY,t(1;9)(p13;q33),9ph. Although, the translocation in the fetus and the father superficially appeared to be identical, the derivative chromosome 9 in the fetus is a recombinant. Most likely, the recombinant chromosome 9 resulted due to crossing over in the inversion loop during the pachytene state. The recombinant chromosome 9 has heterochromatin in both the long and short arms. This finding indicates that that fetus is trisomic for the 9q12-13 region. This pericentric inversion is involved in a rearrangement with breakpoints in the heterochromatic region and presumably has no clinical consequences. We are in the process of characterizing the heterochromatic block by the FISH-technique using alpha, beta and satellite III DNA probes. Reshuffling of various DNA sequences in the qh region have occurred, possibly contradicting the earlier belief that crossing over does not take place in heterochromatin.

Report of a case with manifestations of DiGeorge/Velocardiofacial syndrome (VCFS) and microdeletion 22q11.2 mosaicism. A. Anguiano¹, L. Hudgins², M.B. Dinulos³, L. Mak¹, A. Tayag¹, B.S. Emanuel⁴. 1) Cytogenetics Department, Quest Diagnostics Nichols Inst, San Juan Capistrano, CA; 2) Stanford University School of Medicine, Stanford, CA; 3) University of Washington School of Medicine Children's Hospital & Regional Medical Center, Seattle, WA; 4) The Children's Hospital of Philadelphia, Philadelphia, PA.

A peripheral blood sample from a 12 year-old male was referred for evaluation of DiGeorge/VCFS by FISH. Clinical indications were developmental delay, short stature, mixed hearing loss, probable bipolar disorder, and attention deficit hyperactivity disorder (ADHD). Congenital clubfoot deformity was noted in the patient and several otherwise normal family members. On exam, hypernasal speech, short palpebral fissures, small ears with thickened superior helices and short terminal phalanges were noted. FISH with TUPLE 1 (Vysis) revealed a 22q11.2 deletion in 52 of 107 metaphases (49 %), indicating mosaicism; a deletion was later also demonstrated in 71 of 100 metaphases from skin fibroblasts. FISH studies of 200 metaphases of both parents were normal, indicating post-zygotic, de novo origin of the child's deletion. To determine origin of the deletion, DNA microsatellite analysis of peripheral blood from the patient and parents was done utilizing several markers from within the deletion (D22S941; 102STS). This revealed that the deletion arose on the paternally derived chromosome 22. To further assess the extent of the deletion, FISH studies of G₀ arrested interphase nuclei were undertaken. This revealed mosaicism for a 1.5 Mb deletion. Based on the map of the region, the deletion was determined to involve the proximal portion of the region typically deleted in DiGeorge/VCFS. This report documents a case of 22q11.2 microdeletion mosaicism in a child with predominantly developmental/psychiatric manifestations of DiGeorge/VCFS. This case suggests that mitotic, as well as meiotic recombination events can give rise to the 22q11.2 deletion. It is likely that the duplications (LCRs) on chromosome 22, in particular LCR-A and LCR-B, mediate intrachromosomal somatic recombination events within the modules shared between them.

Tetrasomy 12p Masquerading As Trisomy 21. *L. Dong, R.E. Falk, J. Williams III, M. Kohan, R.R. Schreck.* Medical Genetics-Birth Defects Center, Cedars-Sinai Medical Center, Los Angeles, CA.

CVS was performed on a healthy 38 year old woman at 11 weeks of gestation. Cytogenetic analysis of the direct preparation revealed 5 of 11 cells to have an extra G-group sized chromosome that by G-banding looked like chromosome 21. The direct result was reported as mos 47,XY,+21[5]/46,XY[6]. All 30 cells from two CVS cultures were found to be 46,XY. To clarify the discrepancy between CVS direct preparation and cultures, an amniocentesis was performed at 16 weeks gestation. Ultrasound evaluation was interpreted as normal. However, the amniocentesis result was abnormal with mosaicism for an additional structurally abnormal chromosome. Instead of trisomy 21, an extra small, metacentric chromosome was observed in 12 out of 20 cells. This supernumerary chromosome looked like an isochromosome for either chromosome 21, or for the short arm of chromosome 12. FISH employing a whole chromosome paint for chromosome 12 confirmed the origin of this marker from chromosome 12. To investigate the relationship of this finding to the apparent trisomy 21 cell line in the CVS direct preparation, interphase nuclei from the CVS direct slides were evaluated by FISH using an a satellite probe for chromosome 12. Nine of twenty cells showed three signals. Thus, the extra chromosome seen in the spontaneously dividing cells was derived from chromosome 12, not 21, and could have resulted from breakage of an isochromosome 12p. After termination, a confirmation study revealed 9 of 10 cells from two tissues to have a 47,XY,+i(12)(p10) karyotype. Tetrasomy 12p differs clinically from mosaic trisomy 21, which was suspected based on CVS direct preparation. Tetrasomy 12p is associated with Killian/Teschler-Nicola syndrome which presents with profound mental deficiency, seizures, hypotonia, deafness, coarse facies, and other dysmorphism. While major malformations have been described in prenatally detected cases, ultrasound evaluation of these fetuses is often normal. FISH has proven very useful in defining chromosome material in confusing situations. This case demonstrates the value of molecular cytogenetic tools to clarify and elucidate cytogenetic discrepancies in prenatal diagnosis.

Low level mosaic r(13) resulting in a large deletion of chromosome 13 in a newborn female with multiple congenital anomalies. *C.P. Lorentz¹, D. Babovic-Vuksanovic², D.T. Link³, W. Wyatt¹, S.M. Jalal¹.* 1) Department of Laboratory Medicine and Pathology; 2) Department of Medical Genetics; 3) Department of Otorhinolaryngology, Mayo Clinic, Rochester, MN.

A newborn female had multiple congenital dysmorphic features including: digital anomalies of both hands and feet with severe thumb hypoplasia, facial dysmorphism, agenesis of corpus collosum, bilateral small kidneys, segmental vertebral anomalies, extranumerary rib, heart defect, bilateral hip dislocation, growth retardation and subglottic narrowing. Newborn aneusomy detection based on interphase FISH (NAD) for chromosomes 13,18, 21, X and Y indicated monosomy 13 in 9% of the cells (normal cut off 8.5% at 95% CI). The follow up banded metaphase based analysis indicated the presence of a small ring and monosomy 13 with normal 46,XX cells at a level of 0/20 and 1/50 cells respectively. The ring was wcp13 positive, RB1 negative and was detected in 2/50 cells by metaphase FISH. FISH analysis of buccal cells, based on the level of monosomy 13 where 21 served as a control, indicated the ring to be present in 23% of cells (normal cut off 10%). The low level (< 4%) mosaicism of ring 13 would most likely have been missed if only 20 banded metaphases were analyzed from the whole blood cultures. Fortunately, in this case NAD results alerted us to the loss of chromosome 13 in some cells. Consequently, the analysis of a larger number of cells and the evaluation of multiple tissues established a low level of mosaicism for ring 13 resulting in deletion of most of the 13q arm. The proportion of the ring may be higher in other tissues than observed in the blood. Clinical findings in our patient were consistent with other reported cases of 13q deletions. In addition, our patient had a subglottic narrowing not previously described in patients with 13q deletion.

Identification of an 18q- syndrome patient who is mosaic for two different deletions of 18q. *X.T. Revelles¹, J.D. Cody², N.M. Thompson³, R.J. Leach^{1,2}*. 1) Cellular & Structural Biology, Univ. Texas Health Sci. Center, San Antonio, TX; 2) Pediatrics, Univ. Texas Health Sci Center, San Antonio, TX; 3) Psychiatry and Behavioral Health Sciences, University of Washington, Seattle, WA.

Structural chromosome abnormalities occurring after fertilization result in mosaicism. This results in different populations of cells with the chromosomal abnormality in various tissues. Individuals who are mosaic for chromosomal abnormalities, such as 18q- syndrome, typically have less severe clinical features compared to individuals with a constitutive chromosomal abnormality. We report a child referred to us with a diagnosis of mosaicism for a deletion of the long arm of chromosome 18: 46,XX/46,XX,del(18)(q21.33). However, this child had a more severe phenotype than would be predicted by her breakpoint, irrespective of mosaicism. In order to further define the extent of the mosaicism, samples were obtained from all three cell lineages: leukocytes for mesoderm, urinary tract cells for endoderm, and a buccal smear for ectoderm. A PCR-based marker for the myelin basic protein (MBP) gene was used to analyze the samples and all three cell lineages were hemizygous for the MBP gene (18q23). We were unable to detect any normal cells, which was confirmed by fluorescence in situ hybridization (FISH) in leukocytes. Further molecular analysis was conducted using PCR-based markers D18S51 (18q21.33) and D18S483 (18q21.32). Mosaicism was observed for these markers, which was also confirmed by FISH analysis. Therefore, we determined that the patient has an 18q deletion in all cells, but the deletions have different breakpoints and the child was indeed mosaic. The patient's phenotype was consistent with other individuals with the larger deletion (18q21.32).

Inverted 6p21.3p25p25 duplication in a child with bilateral hearing loss. *D.B. Flannery¹, C.M. Lovell¹, S.M. Jalal², A.S. Kulharya¹*. 1) Department of Pediatrics Medical College of Georgia, Augusta GA; 2) Mayo Clinic Rochester, MN.

Thirty-six cases of partial duplication of short arm of chromosome 6 have been described, however only four have pure duplication of 6p. The remaining cases involve additional deletions from another chromosome, as a result of segregation from balanced reciprocal translocation carriers. We present a rare case of inverted duplication involving 6p21.3-p25. A four-week old infant was evaluated for dysmorphic features. The pregnancy was complicated by oligohydramnios and breech presentation. The birth wt. was 1.32 Kg. Large patent ductus arteriosus and hydrocele were present. Renal sonogram was normal. Thrombocytopenia was present with 20 petechiae. At two weeks all growth parameters were <5th centile. Other features included dolichocephaly, posteriorly rotated ears, prominent forehead, sparse hair, short palpebral fissures, undescended testes, and bilateral hearing loss. Chromosome analysis demonstrated an inverted duplication of 6p21.3p25. FISH with WCP6 confirmed that the duplication was derived from chromosome 6. Analysis of 6p and q-telomere specific FISH probes indicated the presence of the short arm telomere between the terminal end and the duplicated material confirming the inverted duplication. Parental karyotypes were normal. Our patient confirms the phenotype of patients with pure duplication of 6p. The size of duplication does not necessarily seem to correlate to the severity of dysmorphism. The differences in phenotype can be attributed to point mutations, position effect, or parent of origin influence in addition to the size of duplication. The deafness in our patient is a new feature, not previously described. No gene for deafness has been currently mapped in the breakpoints involved in this duplication. Pure duplications involving 6p continue to be very rare, although unequal crossing over involving moderately repetitive sequences are relatively common.

FISH reveals partial trisomy 16p in a fetus with absent thumbs: Implications for genetic counseling and gene discovery. *L.M. Call^{1,2}, J. Baker¹, X. Qian^{1,2}, E. Lemyre^{1,2}*. 1) Brigham and Women's Hospital; 2) Harvard Medical School, Boston, MA.

The study of rare phenotypes may provide important clues about genomic regions containing genes controlling critical aspects of development. Our patient, a 21-year-old female, presented at 13 weeks gestation with abnormal ultrasound findings indicating fetal demise. At autopsy a macerated fetus was noted to have absent thumbs. Due to a family history of SABs, the patient was referred for cytogenetic analysis. Her karyotype revealed a rare balanced 3-way translocation: 46, XX, t(2;16;21)(q13;p13.1;q22.3). Of 20 possible gametes that can result from the 3:3 segregation of this translocation, three will have partial trisomy for 16p13.1-pter with other chromosomal abnormalities. We used targeted FISH on paraffin-embedded tissue of the fetus because routine metaphase analysis was not possible. Cosmids mapping to 16p13.3 were used as probes for FISH; three signals were observed in 47/53 cells analyzed indicating trisomy for 16p13.3. Hybridization of an a-satellite 16 probe detected two signals, demonstrating that fetal demise was not due to full trisomy 16. Hybridization of a cosmid to 21q22.2 also detected two signals. FISH with additional probes for chromosomes 2, 16 and 21 will delineate further the genetic complement of this fetus. Many syndromes feature absent thumbs as a characteristic phenotype, including Fanconi anemia, 13q-, VATER, Baller-Gerold, Holt-Oram, and partial trisomy 16p syndrome. In addition to absent thumbs, individuals with partial trisomy 16p syndrome have developmental delay and a characteristic facies. More than 18 cases of trisomy 16p syndrome have been reported in the literature; at least 6 of these presented with absent or abnormal thumbs. No other imbalance that could result from this 3-way translocation has a known association with absent thumbs. Our findings help explain the etiology of the fetal demise and also have implications in future counseling. In addition, they help to delineate further possible candidate regions for genes involved in limb development.

Clinical and Cytogenetical Features of Partial Trisomy 13q Due to Maternal Pericentric Inversion. *J. D. Falcon, E. Lieberman, M. Cervera, C. Salas, V. Del Castillo.* Dept Human Genetics, Inst Nacional de Pediatra, Mexico, DF, Mexico.

We report the case of a newborn male who clinically presented large fontanelles, short palpebral fissures with bilateral anophthalmia, bushy eyebrows, long eyelashes, prominent nose, low malformed ears, holosystolic murmur, hepatosplenomegaly, fingers and toes with hypoplastic nails and postaxial polydactily of the left foot. He was surgically treated for gastroesophageal reflux and an intestinal volvulus and died at age 2 months due to cardiac and respiratory failure. He was the fifth product of elder parents, had three healthy siblings and one who died in the neonatal period due to unknown causes. Karyotype analysis with G-banding of prometaphase spreads demonstrated a 13p+ chromosome with absent satellites. FISH analysis with painting for chromosome 13 allowed us to determine that the segment was a partial duplication 13 (46, XY dup 13q34q22). The fathers karyotype was normal. The mothers revealed a pericentric inversion involving one of the chromosomes 13 (46, XX inv 13p11.2q22). We could infer that the duplicated segment was due to meiotic recombination of the rearrangement in the mother. Phenotypical characteristics of partial duplications of euchromatic regions on chromosomes can be detected by high resolution and molecular cytogenetic techniques. Karyotype-phenotype correlation can be done for accurate diagnosis and genetic counseling in parents. Partial trisomies of chromosome 13 have been described and characteristic phenotypes have been recognized for proximal and distal segments. Some of these partial duplications arise from chromosomal rearrangements, of which, maternal inversions have been commonly reported. Our patient presented many malformations including anophthalmia, not previously reported, and died at an early age, in contrast to previous reports, which refer to less severe malformations and a longer life span. Though variability of the phenotype exists, this could be explained by the influence of regulatory mechanisms of other genes, inconsistency in the karyotype at the submicroscopic level or a position effect of the segment involved.

A double balanced chromosomal translocation, with development delay and Primary Ciliary Dyskinesia (PCD), suggests candidate genomic regions for PCD loci. *S. Dahoun¹, M.S. Williams², C. Vieux¹, K.D. Josephson², S.E. Antonarakis¹, L. Bartoloni¹.* 1) Division of Medical Genetics, University of Geneva Medical School, Switzerland; 2) Dept. of Pediatrics Gundersen Lutheran Medical Center, La Crosse, WI, USA.

A six year old child was evaluated for development delay and minor dysmorphic features. Additional phenotypic features included rhythmical movement disorder of sleep and speech delay. There was also chronic nasal congestion and recurrent sinus and ear infections. Sweat chloride and immune system evaluation were normal. Electron microscopy of the respiratory cilia revealed absence of both outer and inner dynein arms, absence of radial spokes and fused or single central microtubules, suggesting the diagnosis of Primary Ciliary Dyskinesia (PCD). PCD is characterized by immotile cilia, bronchiectasis and recurrent upper respiratory tract infections. Situs inversus, seen in about 50% of PCD patients, was not present in this patient. Because of the developmental delay, a cytogenetic analysis was performed. A double balanced chromosomal translocation 46,XY,t(7;14)(q32.2;q22.3),t(12;20)(q23.2;p13) was found. Chromosomes of both parents were normal and no familial history of respiratory problems was reported. One of the hypotheses for the PCD phenotype in this patient is that at least one of the breakpoints disrupts a gene coding for an axonemal dynein, and the other allele harbors a deleterious mutation. Mutations in dynein genes (one for an intermediate chain and one for a heavy chain) have been previously shown to cause PCD. Two heavy chain dynein genes map to chromosomal regions 7q32 and 14q. The position of these genes with respect to the translocation breakpoints seen in this patient is now under study. sophie.dahoun@medecine.unige.ch

Trisomy 20 mosaicism-abnormality or normal variant? *M.G. Bialer¹, E. Cox², J.K. Lundberg¹, C. McKenna¹, L. Mehta¹*. 1) Dept Pediatrics, North Shore Univ Hosp/NYU School of Med, Manhasset, NY; 2) Sarah Lawrence College, Bronxville, NY.

A 37 yr old woman had amniocentesis for maternal age. The study revealed trisomy 20 mosaicism (T20mos) with 31 of 33 cells trisomic (47,XX, +20[31]/46,XX[2]). A follow-up ultrasound at 22.9 wk gestation showed the fetus to be 2 wk behind in growth. Fetal echocardiogram revealed tetralogy of Fallot. The couple elected to terminate the pregnancy by D&E. A chromosome study done on kidney from products of conception confirmed T20mos (47,XX,+20[2]/46,XX[5]). T20mos was seen in 6 cases of 13,186 amniocenteses performed at North Shore Univ. Hosp. from 1984-1993, for an incidence of 1 in 2,199. This is, of course, a selected population. We have provided counseling for 13 pregnancies with T20mos and 2 pregnancies with isochromosome 20q mosaicism (i20qmos) since 1984. One pregnancy with both T20mos and T9mos was not included in this study. To our knowledge, all cases resulted in the birth of a normal (nl) baby. We were able to contact all but 2 of the mothers. Nl growth and development were reported in 10 of 11 children with T20mos at the following ages in years: 0.2, 1.6, 1.8, 1.9, 2.3, 3.9, 5.2, 5.7, 5.9, 6.5. Nl growth and development were reported in both children with i20qmos at 1 wk and 3.4 yr respectively. One child with T20mos had nl development until 3 yr when he was noted to have fine motor problems and lost some speech abilities. He was diagnosed with pervasive developmental disorder (PDD). At 9.5 yr his problems include self-stimulatory behaviors, echolalia, hyperacusis, processing problems, short attention span and difficulty relating to others. His growth is nl and he is not dysmorphic. Prenatally diagnosed T20mos has been reported to be compatible with a nl outcome in >90% of cases. The only recurrent anomalies have been congenital heart disease and renal anomalies. There has been little long-term follow-up of children with this diagnosis. Our experience confirms the nl outcome of most cases and the increased risk for congenital heart disease. PDD has not been previously reported with T20mos, and it is possible that this is a chance occurrence, but longer follow-up of children with this diagnosis may be indicated.

Cryptic duplication of 21q in an individual with a clinical diagnosis of Down syndrome and review of the literature. *C.J. Forster-Gibson¹, J. Davies¹, J.J. MacKenzie², K. Harrison³*. 1) Departments of Family Medicine; 2) Pediatrics; 3) Pathology, Queen's University, Kingston, ON.

The clinical features of Down syndrome (DS) have been well described and are identifiable in infancy. We describe an adult male initially diagnosed with Down syndrome at 18 months of age, who had repeatedly normal lymphocyte and fibroblast karyotypes. He had several features characteristic of DS, including mental retardation, 5th finger clinodactyly, open mouth, oblique eye fissures and hypotonia. He did not have Brushfield spots, transverse palmar creases, or congenital heart defect, but did have some dysmorphic features not characteristic of DS. Karyotyping was repeated when he was 33 years old. Analysis of G-banded metaphase chromosomes at the 600 band resolution showed that one chromosome 21 had a possible duplication of 21q22.13 to 21q22.3. Investigational FISH studies were done with a whole chromosome paint probe (wcp21, Oncor Coatasome 21) and a chromosome 21 locus specific cosmid contig probe (LSI-21 (Vysis)) localized within bands 21q22.13-21q22.2. The wcp21 probe hybridized to the entire length of both chromosome 21 homologues. The LSI-21 probe presented as 2 discrete signals on one chromosome 21 homologue and one signal on the other. CGH analysis identified a ratio of 1.5 for part of chromosome 21 involving band 21q22, indicating a gain of part or all of the terminal band of chromosome 21. This was consistent with the conventional cytogenetic and FISH findings. The karyotype was defined as 46,XY,?dup(21)(q22.13q22.2).ish dup(21) (LSI-21++,wcp21+). Review of our case and 12 previously reported cases of pure partial chromosome 21 trisomy indicated that characteristics common to this group included mental retardation (92%), 5th finger clinodactyly (89%), open mouth (88%) and oblique eye fissures (85%). Presence of these features did not appear to depend on the specific portion of chromosome 21 that was trisomic. Features seen frequently in DS (>40%), that were infrequent in this group included a transverse palmar crease (18%) and congenital heart defect (9%). Review of 18 additional DS clinical features showed no consistent phenotype-genotype correlations.

Large Pericentric Inversion of the Y Chromosome in a Severe Oligospermic IVF donor. *L. Bao¹, M. Cedars², D.A. Kelk², T. Gilfillan¹, S.M. Bundrant¹, L. McGavran¹.* 1) Colorado Genetics Laboratory, Depts of Pathology and Pediatrics, University of Colorado Health Sciences Centers; 2) Dept of Obstetrics & Gynecology, University of Colorado Health Sciences Center.

We report a rare pericentric inversion of the Y chromosome in a 43-year-old infertile man with severe oligospermia referred for study prior to using his sperm for in vitro fertilization (IVF). The couple had two previous children by donor insemination prior to the diagnosis of premature ovarian failure in the wife. IVF was planned using donor eggs. The sperm count as assayed in pelleted semen revealed 3 motile and one non-motile sperm. Of 11 mature donor eggs, only 3 were fertilized and 2 embryos were transferred. At 4 weeks post-conception, a singleton fetus with a detectable heartbeat was observed by ultrasonography. The husband's peripheral blood G-banded cytogenetic studies at 650 bands revealed a rearrangement of short arm of the Y chromosome at band Yp11.3. Fluorescence in situ hybridization showed that this arrangement is a pericentric inversion with a large inverted segment and small segments distal to the inversion breakpoints: inv(Y)(p11.31q12). The Yp subtelomeric sequences and the SRY gene (Yp11.3) are inverted to the Yq, and the Yq subtelomeric sequences to the Yp. Such a large pericentric inversion of chromosome Y has not been reported previously, possibly because it is difficult to recognize from chromosomal banding patterns alone. Cases with rearrangements of Yp11.3 have been reported in males with an otherwise normal phenotype and azoospermia. We speculate that this inversion is associated with this man's infertility by altering or interfering with meiotic chromosome pairing and therefore inhibiting spermatogenesis. It is also possible that the inversion may increase the likelihood of unequal recombination between the pseudoautosomal regions of the Xp and Yp, which could result in an XX SRY-positive or an XY SRY-negative karyotype in the offspring. The successful IVF demonstrates that gametes from this man are capable of conceiving a viable embryo. This case illustrates the importance of cytogenetic studies of men with oligospermia prior to utilization of their sperm for IVF.

Clinical studies on subtle chromosomal rearrangements: experiences in the UK. *B.B.A. de Vries^{1,2}, S.M. White³, S.J.L. Knight⁴, T. Homfray⁵, I. Young⁵, B. Kerr⁵, C. McKeown⁵, M. Splitt⁵, O.W.J. Quarrell⁵, A.H. Trainer⁵, M.F. Niermeijer¹, S. Malcolm², J. Flint⁴, J. Hurst³, R.M. Winter².* 1) Clinical Genetics, Erasmus Univ, Rotterdam, The Netherlands; 2) Clinical and Molecular Genetics Unit, Inst of Child Health London; 3) Dept of Clinical Genetics, Oxford Radcliffe Hospital Trust, Oxford; 4) Inst of Molecular Medicine, John Radcliffe Hospital, Oxford; 5) Departments of Clinical Genetics, St George's London, Nottingham, Manchester, Birmingham, Newcastle, Sheffield, Glasgow.

Subtle telomeric defects have been found in 7.4% of children with moderate to severe mental retardation and in 0.5% with mild retardation. To ascertain selection criteria for testing, we studied 29 patients with a known subtelomeric defect and assessed 30 clinical variables concerning birth history, facial dysmorphism, congenital malformations and family history. Controls were 110 undiagnosed mentally retarded children with normal G-banded karyotype and without submicroscopic subtelomeric abnormalities. 41% of the telomere cases had microcephaly compared to 35% of the controls ($p=0.53$), 52% had short stature compared to 32% of the controls ($p=0.07$) and prenatal onset of growth retardation was found in 37% compared to 9% in the controls ($p<0.001$). Other common features in telomere patients were hypertelorism (31%), nasal and ear anomalies (48% resp. 41%), hand anomalies (38%) and cryptorchidism (33%). Two or more facial dysmorphic features were observed in 83% of the patients which was not significantly different from the controls (67%, $p=0.10$). A higher percentage of positive family history for mental retardation was reported in the study group than the controls (50% versus 21%, $p=0.002$). However, miscarriage(s) seems to be less frequent among mothers of telomeric cases than controls (8% versus 30%, $p=0.028$).

Our results suggest that strong indicators for telomeric defects are prenatal onset growth retardation and a positive family history for mental retardation. Using these clinical criteria in addition to features suggestive of a chromosomal phenotype will improve the diagnostic pick up rate of subtelomeric defects among mentally retarded individuals.

Proximal deletion breakpoints in 15q11-q13 are sites of high homologous recombination. *J.L. Gair¹, B.D.*

Kuchinka¹, M. Lalande², R.J. Ritchie³, W.P. Robinson¹. 1) Dept. Medical Genetics, Univ. British Columbia, Vancouver, BC, Canada; 2) Dept. Genetics and Dev. Biol., Univ. Connecticut Health Ctr, Farmington, CT, USA; 3) Research and Productivity Council, Fredericton, NB, Canada.

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are caused by loss of imprinted genes within either maternal or paternal chromosome 15q11-q13, respectively. The most common etiology for PWS and AS is a ~4 Mb *de novo* interstitial deletion of this region. There are two common proximal deletion breakpoints (BP1 and BP2) with similar frequencies of each in both maternally and paternally derived deletions, and one common distal breakpoint. Estimates of meiotic recombination around the proximal deletion breakpoints could not previously be analyzed due to lack of a centromeric marker. Using a new marker (738CA), which lies in a duplicated region proximal to BP1, 370 informative meioses from CEPH pedigrees have now been identified in this region. The genetic distance for males and females from 738CA to D15S541 (BP1) and from D15S541 to D15S11 (BP2) is estimated as 7.8 and 2.2 cM in females and 2.3 and 2.0 cM in males, respectively. Although the exact physical distance is unknown, this data suggests that the proximal deletion breakpoints show a relatively high rate of meiotic recombination, particularly in females. In addition, 14 new microsatellite markers plus additional informative meioses have been used to further characterize the sex-specific regions of recombination previously described in 15q11-q13. The new data supports the presence of excess female recombination extending from D15S122 to D15S97 (including the 5' end of the UBE3A gene) with estimates of 5.0 cM in females vs. 2.7 cM in males, as well as excess male recombination from GABRB3 to D15S156. However, the recombination events do not seem to cluster to a specific site within these regions. Overall the genetic distance from 738CA to D15S165, which spans the ~4Mb deletion region, is estimated as 22.4 cM in females and 20.1 cM in males. This high rate of genetic to physical distance could suggest it is a region either particularly prone to double strand breaks or efficient at pairing during meiosis.

A Unique Finding of Numerical and Structural Three Cell Line Mosaicism Involving Chromosome 18 in a 3 1/2 year old Child. *D.P. Dumont¹, O.T. Mueller^{1,2}, J.R. Ranells², J.A. McFarland¹, F. Mawani¹, D. Conforto¹, M.J. Sutcliffe^{1,2}.* 1) Clinical Genetics Laboratories, All Children's Hospital, St Petersburg, FL; 2) Department of Pediatrics, University of South Florida, Tampa, FL.

A term normal birthweight female born to a 47 year old para 1-0-0-1 mother was diagnosed with supravulvar pulmonary stenosis. Chromosome analysis at a different facility revealed a 47,XX,+del(18)(q22)[18]/47,XX,+18[2] karyotype. Additional clinical presentation included an MRI finding of hypoplastic brain stem with large cisterna magna, apnea, seizures, mild dysmorphic features and a few scattered nevi flammei. Following excellent family care and intensive stimulation, current clinical evaluation demonstrates both psychomotor and physical progress despite growth delay, microcephaly and hypotonia. Due to tissue specific mosaicism, a skin biopsy was performed at 3 1/2 years. A centromeric Fluorescence In-Situ Hybridization (FISH) probe, CEP18, revealed a normal hybridization pattern in 7/100 cells. Fibroblast GTG banding confirmed 46,XX[4] normal cell line with 47,XX,+del(18)(q22)[32]/47,XX,+18[14]. Repeat peripheral blood analyzed by CEP18 FISH showed 39/200 cells with a normal hybridization signal. Further FISH analysis using dual color subtelomeric probes for 18p and 18q showed 8/100 cells with a normal hybridization pattern, 6/100 with +18 and 73/100 with the del(18). Karyotype showed 46,XX[1]/47,XX,+del(18)(q22)[99]. DNA microsatellite analysis using centromerically oriented loci established the maternal origin of the additional chromosome 18, and suggested a meiosis I nondisjunction event. Further, using loci distal to the q22 breakpoint established that the deleted chromosome was the remaining paternal 18. This case illustrates the importance of taking counts above 20 cells in known or suspected mosaicism, since missing the normal cell line has prognostic implications. In addition, this case demonstrates both numerical and structural abnormalities and the limitations of the initial trisomy 18 analysis using a FISH centromeric probe for 18.

Full term pregnancy after diagnosis of non-mosaic trisomy 10 by amniotic fluid: ultrasound, cytogenetic and morphologic findings. *S.A. Ebrahim¹, A.N. Mohamed¹, H. Lee², F. Qureshi¹, A. Johnson², M.I. Evans^{1,2}.* 1) Department of Pathology; 2) Department of Obstetrics/Gynecology, Hutzel Hospital/Wayne State University, Detroit, MI.

Non-mosaic trisomy 10 is a lethal condition seen in about 1.8 per cent of spontaneous miscarriages. We report the ultrasound, cytogenetic and morphologic findings of a fetus with non-mosaic trisomy 10 detected at 18 weeks of gestation that continued to full-term.

A 40-year-old gravida 5, para 3, VIP 1, female was referred for genetic counseling and amniocentesis due to anomalies detected on a routine ultrasound scan and advanced maternal age. Ultrasound revealed a growth-retarded fetus (16 weeks by ultrasound and 18.5 weeks by LMP date). Multiple anomalies were noted, including absent lower cerebellar vermis, dilated frontal horns of the cerebral ventricles, enlarged thalamus, pericardial effusion, bilateral pleural effusion, clenched hands with flexed wrists, decreased amniotic fluid volume, and a non-septated cystic hygroma. FISH on uncultured amniocytes showed no evidence of numerical abnormality for chromosome 13, 18, 21 X, and Y. Cultured amniocytes showed a 47,XX,+10[20]. The couple was counseled regarding these findings, and opted to continue the pregnancy. At 42 weeks, a stillborn female fetus was delivered. At birth, the fetus weighed 1480 gm and was found to have multiple dysmorphic features and congenital anomalies, including hypertelorism, bilateral microphthalmia, right cleft lip and palate and left cleft palate, posteriorly rotated ears, thymic hypoplasia, multicystic dysplastic kidneys, streak maldeveloped ovaries and splenomegaly. Examination of the cardiovascular system revealed interrupted aortic arch, absent ductus arteriosus and subaortic ventricular septal defect. To our knowledge, this is the first reported case of non-mosaic trisomy 10 detected by amniocentesis early in gestation which survived to full term.

Interpreting Comparative Genomic Hybridization Data in Clinical Cytogenetics. *T.M. Dunn, N.B Kardon, K. Hirschhorn, B. Levy.* Depts Human Genetics and Pediatrics, Mount Sinai Medical Ctr, New York, NY.

Comparative genomic hybridization (CGH) is an alternative molecular cytogenetic technique that can characterize unbalanced G-banded material in a one-step global screening procedure. The advantage of CGH over conventional FISH with whole chromosome paints (wcps) and multicolor FISH is its ability to identify not only the chromosome from which the additional unknown material was derived but also to map the region involved to specific bands on the source chromosome. Defining the origin of additional cytogenetic material by FISH with various probes is expensive and laborious as numerous wcps may be required until the source chromosome is identified. To date, more than 430 articles have been published on CGH with ~80% reporting the utility of CGH for analysis of cancer specimens. Technical aspects comprise the majority of the remaining papers and only a limited number have described the application of CGH in a clinical cytogenetics setting. CGH software is now available as an option on many FISH imaging systems without the need for additional equipment. With many laboratories acquiring image analysis equipment for routine cytogenetic and FISH analysis, the utilization of CGH as a tool in clinical cytogenetics is likely to increase. Since 1996, we have performed CGH analysis on 45 postnatal and 6 prenatal clinical samples received from our institution and from thirteen other medical centers. In all of these cases, traditional cytogenetic analyses revealed aberrant chromosomal material of unresolved origin. These samples have included markers, unbalanced translocations, duplications, deletions and inversions. More than 50% of these cases were subjected to multiple wcps and locus specific probes by the referring institutions before being sent to our laboratory for CGH analysis. The results obtained by CGH analysis proved effective in revising the karyotype in all cases. CGH was performed on aberrations which varied extensively in size and thus provided a realistic idea of the minimal size of duplication/deletion detectable by CGH. In this report, we review our experience, detailing the benefits and limitations of CGH in a clinical cytogenetic setting.

Partial duplication of chromosome 6 associated with obesity and hypogonadism. *I.K. Gadi¹, L. Kirsh², P.N. Mowrey¹, P.R. Papenhausen¹, J.H. Tepperberg¹, K. Phillips¹, R. Johnsonbaugh²*. 1) Dept Cytogenetics, Laboratory Corp of America, Res Triangle Pk, NC; 2) Arizona Pediatric Endocrinology, Phoenix, AZ.

A 16 year old Hispanic boy with normal perinatal and neonatal periods (birth weight 3500 gm, length 53 cm and normal discharge from the hospital), mild intellectual delay, emotional impairments, obesity (>95%tile), normal height (75 %tile), absent midline cranial defects, gynecomastia, hypogonadism (testicular volume 4 mL bilaterally, pubic hair Tanner early stage II, mild amount of axillary hair, breasts Tanner stage 4 with a bone age of 16 years) of central origin (testosterone 81 ng/dL, LH<1uIU/ml and FSH<1IU/ml) with normal ability to smell was referred for chromosome analysis. High-resolution chromosome analysis revealed an apparent partial duplication of the long arm of chromosome 6. Fluorescence in situ hybridization (FISH), using a whole chromosome 6 paint probe confirmed chromosome 6 derivation. The G-band pattern suggests a duplication of the q23-q24 region. Previously reported paternal duplications of long arm chromosome 6 or uniparental disomy of paternal chromosome 6 have been associated with transient neonatal diabetes mellitus. Our patient with unremarkable perinatal and neonatal periods is unique. Microsatellite molecular analysis of proband and parental DNA will be pursued to determine the parent of origin of the duplication and the extent of duplication.

Recombination in the pseudoautosomal region in a 47, XYY male. *R.H. Martin*^{1,2}, *Q. Shi*^{1,2}. 1) Medical Genetics Clinic, Alberta Children's Hosp, Calgary, AB., Canada; 2) Department of Medical Genetics, University of Calgary, AB, Canada.

Recombination between the X and Y chromosomes primarily occurs in the pseudoautosomal region and is thought to be necessary for proper segregation of the sex chromosomes during spermatogenesis. Failure of the sex chromosomes to disjoin properly during meiosis can result in individuals with a sex chromosomal aneuploidy. Males with a 47, XYY karyotype generally have chromosomally normal children, despite the high theoretical risk of aneuploidy. Also, studies of sperm karyotypes or FISH analysis of sperm have demonstrated that the majority of sperm are chromosomally normal in 47, XYY men. Our results on FISH studies in two 47, XYY males indicated a frequency of gonosomal abnormalities of approximately 1%. There have been a number of meiotic studies of XYY males attempting to determine if the extra Y chromosome is eliminated during spermatogenesis, with conflicting results regarding the pairing of the sex chromosomes and the presence of an extra Y. We have analyzed recombination in the pseudoautosomal region of the XY bivalent to determine if this is perturbed in a 47, XYY male. A recombination frequency similar to normal 46,XY men would indicate normal pairing between the XY bivalent, whereas a significantly altered frequency would suggest other types of pairing such as a YY bivalent or XYY trivalent. Two DNA markers STS/STS pseudogene and DXYS15 were typed in sperm from a heterozygous 47, XYY male. Individual sperm (23, X or Y) were isolated using a FACStar^{Plus} flow cytometer into PCR tubes. Hemi-nested PCR analysis of the two DNA markers was performed to determine the frequency of recombination. A total of 108 sperm were typed. The frequency of recombination between the two DNA markers was 38%. This is very similar to the frequency of 38.3% that we have observed in 329 sperm from a normal 46, XY male. Thus, our results suggest that XY pairing and recombination occur normally in this 47, XYY male. This could occur by the production of an XY bivalent and Y univalent (which is then lost in most cells) or by loss of the extra Y chromosome in some primitive germ cells or spermatogonia and a proliferative advantage of the normal XY cells.

Partial trisomy 4p and partial monosomy 13q in a patient with pan-lymphopenia. *L.H. Lockhart, G.V.N. Velagaleti, F.C. Schmalstieg, A.S. Goldman.* Dept. Pediatrics, University of Texas Medical Branch, Galveston, TX.

A six-year old male presented with dysmorphic features, mental retardation, developmental delay and an increased frequency of respiratory infections. He also had a persistent lymphopenia ($1000-1200/\text{mm}^3$). Chromosome analysis showed an unbalanced translocation involving chromosomes 4 and 13 [46,XY,+der(4)t(4;13)(q12;q12),-13], resulting in partial trisomy 4pter-q12 and partial monosomy 13pter-q12. FISH studies with alpha satellite probes D4Z1 and D13Z1/D21Z1 showed that the unbalanced karyotype resulted from an "adjacent-2" segregation of a maternal balanced translocation. The translocation is known to be segregating for three generations in the family. As shown by flow cytometry, the major blood lymphocyte populations were reduced in number, where as other hemapoietic lineages were normal. Blood T-cells were principally CD45RA+, CD62L+ and deficient in the Fas receptor. Serum concentrations of IgG (632 mg/dl), IgA (106 mg/dl), and IgM (41 mg/dl) were all normal. The serum titers of IgG, IgA antibodies to tetanus and diphtheria toxoids rose significantly after immunization, but only IgM antibodies appeared in response to the first immunization with penumococcal polysaccharides. Serum IgG and IgA antibodies to these polysaccharides appeared, however, after second immunization.

The pan-lymphopenia may be due to an overdose of a gene or genes located in the region of chromosome 4 or a partial deficiency of a gene or genes in the region of chromosome 13 that regulate the development of the lymphocyte lineage. Since the mother contributed two copies of chromosomal region 4pter-q12 and no copy of 13pter-q12, the pan-lymphopenia in our patient could be the result of either uniparental disomy or imprinting.

A rec(4) dup 4p inherited from a mat inv(4)(p15q35). Case report and review. *J.A. Martin¹, W. Chiu², M.F. Garcia², J. Garcia-Heras².* 1) Prescott, AZ; 2) Genetic Testing Center, TDH, Denton, TX.

Rec(4) dup 4p is a rare rearrangement that is usually the product of a parental pericentric inversion of chromosome 4 that results in a duplication of 4p and a deletion of 4q distal to the inversion breakpoints. We present a new case and review 7 previous reports.

A 4-year-old girl received genetic evaluation due to developmental delay, lack of speech, severe behavioral problems and malformations. A physical exam showed microcephaly, short neck, broad chest with widely spaced nipples, hypertonia and craniofacial dysmorphism. Her height was 92.5 cm. (5%), weight 6.3 kg (50%) and OFC was 48 cm. (5th%). A cardiac exam revealed a ductus arteriosus and pulmonary hypertension. A cytogenetic study in lymphocytes demonstrated a 46,XX, rec(4) dup 4p that was inherited from a maternal inv(4)(p15q35). The father was normal and two other healthy sibs carried the same pericentric inversion detected in the mother.

The manifestations of this child resemble trisomy 4p like 3 other patients (craniofacial and chest anomalies in both sexes and abnormal genitalia in males). In the other 4 cases the spectrum of malformations is more variable. In either group the phenotype is not specific, and a diagnosis is not feasible without cytogenetic studies. This lack of a clinically recognizable phenotype could reflect the effects of the variable size of the deletion of 4q, molecular differences in breakpoints and/or the known variable expression of trisomy 4p. Most rec(4) dup 4p derived from familial pericentric inversions with breakpoints at 4p13p15 and 4q35 and are paternal in origin (5/8 cases). A carrier of this inversion could have with equal frequency offspring with the 2 alternate duplication/ deletion recombinants due to the size of the inverted segment. But the rec(4) dup 4p is more frequent (7/9 cases) so either the rec(4) dup 4q is less viable or in these inversion carriers, mostly males, meiotic recombination favors the generation of rec(4) dup 4p. The latter hypothesis could be tested in male carriers with the sperm penetration hamster assay.

Phenotype-genotype correlation in 7 patients with 5p/autosome translocations. Risk for carriers of translocations involving 5p. *P.C. Mainardi¹, A. Cali^{1,2}, G. Guala¹, C. Perfumo², M.E. Liverani¹, G. Pastore¹, J. Overhauser³, F. Zara², M. Pierluigi², F. Dagna Bricarelli².* 1) Divisione di Pediatria, Servizio di Genetica, Ospedale S Andrea, Vercelli, Italy; 2) Laboratorio di Genetica Umana, E.O. Ospedali Galliera, Genova, Italy; 3) Department of Biochemistry and Molecular Biology, Thomas Jefferson Medical College, Philadelphia, PA, U.S.A.

Seven patients with 5p/autosome translocations were identified during a study of 80 CdC patients in the Italian CdCS Register (198 subjects). Four (5%) presented a de novo translocation while three (3,75%) had a familial translocation. All the patients lacked both the critical regions for cat like cry in 5p15.3, as well as that for dismorfisms and mental retardation in 5p15.2. In two patients with de novo translocation the trisomic portion had no phenotypic effect. The study of the correlation in the other 5 patients showed a prevalence of CdCS phenotype with severity associated with deletion size. Where the chromosome involved was 3p or 9p, the phenotype was hybrid because of the effect of the trisomic segment. The analysis of these patients supports the hypothesis that duplications of 9p22.24 and 3p25.26 are critical, respectively for the trisomy 9p and 3p syndromes. This study confirms that the patients with translocations had more severe phenotypes for malformations, morbidity, and psychomotor development delay than those with terminal deletions. The reproductive risk for carriers of translocations involving 5p were defined by the evaluation of personal and reviewed data from 54 pedigrees. The risk of unbalanced offspring according to pachytene configuration and 5p breakpoint localisation ranges from 10.5% for translocation predisposed to 3:1 disjunction to 18.9% for translocation with breakpoint in 5p15 predisposed to adjacent-1 2:2 disjunction. In families referred for multiple abortions, unbalanced offspring were never observed, suggesting that all unbalanced segregations were lethal. With the exception of translocations predisposed to 3:1 disjunction, risks for males and females are similar. Work funded by Telethon-Italy and the Italian Cri du Chat Childrens Association.

An unusual mosaic karyotype derived from a familial t(11;22) in a male with developmental delay. *A.S. Kulharya, C.M. Lovell, D.B. Flannery.* Dept Pediatrics, Medical Col Georgia, Augusta, GA.

Constitutional t(11;22) is the most common recurrent translocation in humans. It is not confined to one particular race or geographic region. The majority of offspring of carriers of the familial translocation inherit either a balanced translocation or an unbalanced karyotype. The abnormal karyotype has 47 chromosomes with der(22) in addition to the normal 46 chromosomes resulting from a 3:1 segregation. We present a developmentally delayed male with an unusual mosaic karyotype derived from t(11;22). He has two sisters who are known carriers of this translocation. The patient is a 26 year old male with developmental delay. The prenatal history revealed considerably diminished fetal movements prior to his birth. He was born with a Pierre Robin sequence and a second curled toe. He is non-communicative and has temper tantrums.

Chromosome analysis of lymphocytes showed a normal male karyotype in all 200 metaphases examined. However, chromosome analysis of fibroblast cells showed a mosaic karyotype: 46,XY[144]/46,XY,der(22)t(11;22)(q23;q11.2)[6]. The der(22) syndrome with a characteristic phenotype is the most common unbalanced consequence from a familial t(11;22). Recently, there have been reports that suggest other forms of unusual segregation for this translocation. In some of these cases the abnormal segregation is a result of either meiosis II or postzygotic nondisjunction of the der(22) chromosome. Our patient provides evidence of yet another form of segregation for t(11;22) that is most likely not viable if present in a non-mosaic form. This form of adjacent-I segregation results in partial monosomy for a significant segment of chromosome 22 and partial trisomy for the distal segment of chromosome 11. The mosaic karyotype in our patient could be a result of a series of postzygotic rescue efforts of a zygote carrying the der(22) chromosome. It is also possible that the patient is a chimera resulting from early fusion of two zygotes; one normal and the other abnormal carrying 46 chromosomes with only the der(22).

A chromosomal rearrangement causing velopharyngeal insufficiency and developmental delay. *W.S. Kerstjens-Frederikse, B. Leegte, J.C. Oosterwijk, E. Vogel, A.J. van Essen.* Dept Med Genetics, Univ Groningen, Groningen, Netherlands.

A 4 year old girl was seen at the Genetics Department because of VPI and developmental delay. The parents were healthy and non-consanguineous. Family history was non-contributory. Motor development was slightly delayed, speech was delayed and hypernasal. Height, weight and skull circumference were normal. The face was not characteristic for velocardiofacial syndrome. The face was triangular and showed telecanthus (ICD 3.3 cm, OCD 8.5 cm). The eyebrows showed lateral flaring and the eyelashes were long and curly. The mouth was small. She had persistent fetal fingerpads. She had a clinodactyly of the 4th finger left. Her movements were jerky with normal reflexes and she showed slightly ataxic gait. She had no signs of a cardiac or renal defect. VPI is seen in approximately 30 % of children with microdeletion 22q11.2 (velocardiofacial syndrome), but this girl did not show other characteristic features of VCF, besides the developmental delay. Chromosomal analysis showed 45,XX,der(13)inv(13)(p10q22)t(13;14)(q22;q11~12). No microdeletion 22q11.2 was found with FISH. A pericentric inversion and balanced translocation was found in the mother: 46,XX,inv(13)(p10q22)t(13;14)(q22;q11~12). The grandparents showed normal karyotypes, so the balanced translocation and inversion was de novo in the mother.

Detailed FISH analyses of Silver- Russell Syndrome (SRS) patients with cytogenetic disruptions of chromosome 7p11.2-p13 define a candidate region for SRS. *D.N. Monk¹, M. Hitchins¹, M. Wassal², K. Temple³, A. Sharp⁴, M. Preece⁵, P. Stanier¹, G. Moore¹.* 1) Fetal and Maternal Medicine, Imperial College School of Medicine, Queen Charlottes and Chelsea Hospital, London, England; 2) Clinical Genetics, National Research Centre, Cairo, Egypt; 3) Wessex Clinical Genetics Services, Southampton University Hospital NHS Trust, Princess Anne Hospital, Southampton, England; 4) Wessex Regional Genetics Laboratory, Salisbury Health Care Trust, Odstock, England; 5) Institute of Child Health, University College London, London, England.

SRS is a congenital disorder primarily involving lateral asymmetry and both intrauterine and post-natal growth restriction. This disorder is genetically heterogeneous, but maternal uniparental disomy for chromosome 7 (mUPD7) has been demonstrated in approximately 7% of cases. Consistent heterodisomy for the full length of chromosome 7 in five mUPD7 subjects indicates that at least one gene on this chromosome is imprinted and involved in the pathogenesis of SRS. We have previously reported a de novo duplication of 7p11.2-p13 on the maternal homologue in a patient with classical SRS features. This duplication was shown to include the GRB10, IGFBP1 and IGFBP3 genes, but not EGFR. A similar case of a maternally inherited duplication of 7p11.2-p13 has also been described by another group. These two cases suggest that SRS may result from over-expression of a maternally expressed gene involved in growth suppression. Mice with maternal disomy for proximal chromosome 11, the orthologous region to human 7p11.2-p13, demonstrate prenatal growth failure, further implicating this region in SRS. We have performed more detailed fluorescent in situ hybridisation analysis on the two patients with duplications to locate the breakpoints more precisely. In addition, we describe a group of SRS patients with other cytogenetic abnormalities involving 7p11.2-p13. The breakpoint mapping data presented here define a candidate region for genes which may contribute to the SRS phenotype in patients with a genetic aetiology involving chromosome 7.

Tightly clustered 11q23 and 22q11 breakpoints permit PCR based detection of the recurrent constitutional t(11;22). *H. Kurahashi, T.H. Shaikh, E.H. Zackai, L. Celle, D.A. Driscoll, M.L. Budarf, B.S. Emanuel.* Div Human Genetics, Children's Hosp Philadelphia, Philadelphia, PA.

The constitutional t(11;22)(q23;q11) is the most frequently occurring non-Robertsonian translocation. The breakpoint of the t(11;22) has been identified within palindromic AT rich repeats (PATRRs) on chromosomes 11q23 and 22q11. These PATRRs are predicted to induce genomic instability which mediates the translocation. To examine the role of the PATRRs in the recurrent rearrangement, a PCR-based translocation detection system for the t(11;22) has been developed. PCR primers were designed in regions flanking the PATRRs of both chromosomes. Forty unrelated t(11;22) balanced carriers plus two additional, independent cases with the supernumerary-der(22) syndrome were analyzed to compare their translocation breakpoints. Similar translocation specific junction fragments were obtained from both derivative chromosomes in all 40 t(11;22) balanced carriers, and from the der(22) only in both of the unbalanced supernumerary-der(22) syndrome offspring. This indicates that the breakpoints of all cases localize within these PATRRs, suggesting that the translocation is generated by a similar mechanism in all cases examined. In spite of the fact that five of the balanced carriers, including two de novo cases, have an abnormal phenotype, their breakpoints also localize within the same PATRRs. Previously, t(11;22) carriers have been reported to be at increased risk for breast cancer. In one family, a balanced carrier with breast cancer was studied. Her breakpoint is also located within the same PATRRs. Since we have previously shown that no genes on 11 or 22 are disrupted by the translocation, neither the abnormal phenotype nor the breast cancer in some t(11;22) carriers appears to be directly related to the translocation itself. It is likely that the palindromic ATRRs produce unstable DNA structures in 22q11 and 11q23 that are responsible for the recurrent t(11;22) translocation. This PCR strategy provides a convenient technique for rapid diagnosis of the translocation, indicating its utility for prenatal and preimplantation diagnosis in families with balanced carriers.

Analysis of genomic instability in Rothmund-Thomson Syndrome. *J.A. Lane¹, L. Wang², S.E. Plon², S.L. Wenger¹.*
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Rothmund-Thomson Syndrome (RTS) is a rare, autosomal recessive disorder characterized by poikiloderma, skeletal abnormalities and an increased cancer risk. Low level trisomy 8 mosaicism, clonal abnormalities and evidence of genomic instability has been reported in some RTS patient, however these findings have been inconsistent. The defect in the helicase gene (RecQL4) at 8q24.3 has recently been identified in some but not all RTS cases. Lymphoblast cell lines established from 2 RTS patients and one control were karyotyped. One patient was from a family discordant for an intragenic SNP at RecQL4 and the other family was concordant. The RTS cell from the discordant family had a karyotype of 46,XY, t(13;14)(q22;q32)[27]/46,XY,del(6)(q25)[5]/46,XY[68]. The other two cell lines had normal karyotypes. To identify low levels of trisomy 8 mosaicism an alpha-satellite FISH probe for chromosome 8 (D8Z2) was used to score 1000 interphase cells for each of the cell lines. 1% or fewer of the cells from all cultures had three chromosome 8 signals, ruling out low level mosaicism. No increase in spontaneous breakage was seen among 100 solid stained metaphase cells for the three cell lines. Although further studies are necessary, our results suggest that genomic instability may not be characteristic of cells from patients with RTS.

Mapping phenotypes associated with 20p deletions. *A. Laufer-Cahana, J.J. Dowhanick-Morrisette, L.D. Bason, E.H. Zackai, N.B. Spinner, I.D. Krantz.* Division of Human Genetics, Children's Hospital of Philadelphia, PA.

The correlation of chromosomal deletions with clinical abnormalities allows for the genomic localization of genes responsible for clinical traits. Patients with deletions of 20p have a number of characteristic features, including Alagille syndrome (AGS), developmental delay (DD), hearing loss, cleft lip and palate, neural tube defects, hypoglycemia and bowel anomalies. We have identified 13 patients with variable deletions of the short arm of chromosome 20. Eight are cytogenetically visible and 5 are submicroscopic. Eleven patients have deletions that include the JAG1 gene and have some features of AGS. Additional phenotypic findings include hearing loss (4/13), cranial nerve palsies (3/13), palatal clefts (2/13) and DD (8/13). One patient has Hirschsprung disease and another has spina bifida occulta. The two patients with the most proximal deletions have unusual developmental features, "savant characteristics" in one and autism in the other. Parental origin could be studied in 9 patients, and the deletions are familial in 3 and de novo in 6 patients. The clinically normal parent of one child with a 20p12 deletion was found to be mosaic, with 9/20 cells from peripheral blood demonstrating the deletion. Review of the literature revealed an additional 18 patients with 20p deletions. Review of phenotypic features in the 31 patients (13 in this study and 18 from the literature) reveals that 29/31 have features of AGS, 26/31 have DD, 9/31 have hearing loss, 5/31 have cleft lip/palate, 4/31 have hypoglycemia and 3/31 have spina bifida. Overall, the frequency of deletions of JAG1 in AGS is relatively low (approximately 3-5%). However, the presence of any of these additional features in an individual with clinical manifestations of AGS (Alagille plus), suggests that cytogenetic analysis should be a first line diagnostic approach. Using a panel of YACs and microsatellite markers the boundaries of the deletions in our 13 study patients are being mapped. We hypothesize that these studies will lead to the identification of critical regions within which genes important in the etiology of hearing loss and cleft lip and palate can be isolated.

Non-invasive cytogenetic analysis in children: Micronuclei in exfoliated buccal and urothelial cells. *N.T. Holland¹, L. Schumacher², L. Gunn¹, A. Hubbard¹, B. Nguyen¹, D. Golden¹, P. Duramad¹, M.T. Smith¹.* 1) Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley, CA; 2) Children's Hospital Oakland Research Institute, Oakland, CA.

Children may be more susceptible to the effects of environmental pollution than adults. While all children are sensitive to environmental hazards, minority children and children of lower socioeconomic status are particularly at risk because they tend to live in less healthy environments where they may be affected by both pollution and inadequate diet. However, few studies have examined biomarkers of genetic damage in children. For community-based population studies involving children, it is essential to develop non-invasive methods of collecting samples. Exfoliated epithelial cells from the mouth and bladder can be easily collected and subjected to cytogenetic analysis as well as analysis of DNA-polymorphisms. The objective of this study was to collect data on the background levels and variability of a spectrum of molecular and cytogenetic biomarkers of exposure, effect, and susceptibility. Our first step was the validation of cytogenetic markers in children using micronucleus assay. Micronucleus frequency is a sensitive measure of genetic damage in vitro and in vivo which reflects chromosome breakage and aneuploidy. In collaboration with the Children's Hospital Oakland, CA, buccal and urothelial cells have been collected from 81 healthy African-American children, ages 5-12, and 49 parents/guardians from the inner city East Oakland, CA. Here, data are presented on cytogenetic biomarkers, particularly micronuclei in two types of exfoliated cells. Children had slightly lower levels of micronuclei than adults in both buccal and urothelial cells ($P < 0.01$). Moreover, an age-dependent change in micronuclei in urothelial cells of children was observed ($P < 0.001$). The frequency of micronucleated cells was slightly higher in urothelial cells of children than buccal cells ($P < 0.001$); but the same was not true for adults. Further studies of the effects of various genetic, dietary and environmental factors on the level of cytogenetic damage in exfoliated cells of inner city children are underway.

Repeat karyotypic analysis may be periodically indicated. *R. Naeem, G.M Cohn, D.J Carneiro, J.G Habecker-Green.* Baystate Medical Ctr, Springfield, MA.

We present 3 case reports of patients whose clinical diagnosis was confirmed on the basis of repeat karyotype analysis.

Patient 1 presented at age 2 years 10 months for a suspected dwarfing syndrome. She was noted to be dysmorphic, and had developmental delay, VSD, and partial cutaneous syndactyly of all digits. Chromosomes previously done at another laboratory in 1992 were reported as normal. Features were not clearly consistent with any known genetic condition. The patient was seen again at age 8 years 4 months for an endocrinology evaluation; that service ordered chromosome analysis. The revised karyotype was 46,XX,del(11)(q23) ish del(11)(tel 11q-). The patient was seen again, and findings are consistent with Jacobsen syndrome. To make this diagnosis repeat karyotype and FISH analysis were instrumental.

Patient 2 presented at age 15 years 8 months for developmental delay. He had a history of surgery for metopic craniosynostosis, glaucoma, inguinal hernia, and umbilical hernia. Family history was remarkable for 2 anomalous maternal uncles who had died. The child had mental retardation, dysmorphic features, and broad thumbs with spoon shaped nails. Chromosomal analysis prenatally had been reported normal at another laboratory. Repeat chromosomal analysis was notable for an unbalanced translocation 46,XY,der(9)t(8;9)(q24;p22) mat. ish der (9)t(8;9) (wcp8+,wcp9+).

Patient 3 presented at age 4 years with a history of congenital microcephaly, growth retardation, and developmental delay. Routine chromosomes were reported normal. She was not found to have features consistent with any genetic syndrome. The patient returned to genetics at 8 years 5 months of age. Based on clinical findings a diagnosis of trisomy 9 mosaicism was suspected. Repeat chromosomal analysis with mosaicism studies were ordered, and revealed low level trisomy 9 mosaicism.

These patients suggest that periodic repeat karyotype may be indicated if a clinical diagnosis remains undetermined or chromosomal anomaly is suspected.

Case report and literature review: Male with apparent unique chromosome 5 double deletion at 5q10q11.2 and 5q14.2q14.3. *H.L. Hansen, W.S. Surka, J.L Zackowski, V.K. Proud.* Dept. of Ped., Div. of Med. Gen., Children's Hospital of The King's Daughters, Eastern Virginia Medical School, Norfolk, VA.

After extensive literature review we report the first case with an apparent chromosome 5q double deletion, 46XY,der(5)del(5)(q10q11.2) del(5)(q14.2q14.3). Case Report: JR was a 25 year old white male with short stature, coarse facial features, deep set eyes, epicanthal folds, strabismus, long eyelashes, ptosis, down-slanting palpebral fissures, hypoplastic maxilla, beaked nose, high arched palate, and low set, malformed auricles. Skeletal findings included marked cervical kyphosis, thoracolumbar scoliosis, asymmetric chest, broad thumbs, and overlapping toes. He had moderate mental retardation. His medical history included recurrent respiratory infections, dysphagia, gastroesophageal reflux, congenital right hydronephrosis with tortuous ureter, nephrectomy for dysplastic kidney, and hernia repair. Laboratory Studies: because the phenotype was similar to Rubinstein-Taybi syndrome additional FISH studies on chromosome 16 were performed but were negative. In hundreds of reported cases with 5q rearrangements, only 16 with interstitial deletions of 5q were cited by Kobayashi et al. [1991]. We were unable to identify any case with a breakpoint at 5q10. At least 6 shared a common breakpoint at 5q11.2 and at least 6 had deletions that include the region 5q14.2-q14.3. Detailed analysis of physical and cytogenetic findings in these 12 cases revealed that the 6 cases with rearrangements, inversions, duplications, and deletions involving the common breakpoint 5q11.2 had renal anomalies, mental retardation, prominent forehead, and malformed ears like JR. The 6 cases with deletions of 5q13q15 had low-set, malformed ears, mental retardation, short neck, short stature, and down-slanting palpebral fissures like JR. Tissue samples of this unique double deletion in 5q are available for molecular studies and genotype/phenotype correlation.

Delineation of trisomy 16q resulting from de novo X:16 translocation by cytogenetic, FISH and late replication studies in a child with mild dysmorphic features - A case report. *F.M. Mohammed¹, J. Tolmie², J.M. Connor², L. Bastaki¹, D.S. Krishna Murthy¹.* 1) Cytogenetics Laboratory, Kuwait Medical Genetics Center, SULAIBIKAT P.O., KUWAIT, KUWAIT; 2) Duncan Guthrie Institute of Medical Genetics, Glasgown, UK.

We report an 18-month-old female child with dysmorphic features, heart defect, and developmental delay. Routine chromosome analysis using G-banding technique showed an abnormal X chromosome (Xp+). Karyotype of the parents was normal. Application FISH technique using whole chromosome paint and centromeric probes, chromosome arm specific probes (CAP) and chromosome library probes for all autosomes and sex chromosomes (DXZ-1 Oncor; WCP-Cambio; CAP- Biomedical Research Corporation; Cytocell Multiprobe Kit) confirmed an unbalanced between the short arm of X chromosome (Xp22.3) and long arm of chromosome 16 resulting in trisomy 16q (q24). The karyotype is interpreted as: 46,X, -X,+der(X), t(X;16)(p22.3;q24). Late replication studies showed pale staining of the abnormal der X chromosome with the dark band region of the 16q segment in most of the cells, confirming late replication of the abnormal X with an active segment of 16q. The occurrence of balanced/unbalanced structural anomalies involving X autosome translocations is very rare. They are associated with normal to severe pathogenesis, depending on the chromosomes involved and the extent of monosomy/trisomy of the segments involved. Inactivation and spreading effect may also contribute to the severity of the phenotype and pathogenesis. Application of FISH using multiprobe kit enabled in delineating this cryptic de novo X-autosome translocation precisely.

Study of aneuploidy rate of sperm in a man heterozygous for a t (1,10) (p25 ; q32) reciprocal translocation by FISH. *N. Miharū, M. Yoshimoto, Y. Ohashi, H. Honda, K. Ohama.* Dept OB/GYN, Hiroshima Univ Sch Medicine, Hiroshima, Japan.

In order to quantify the relative proportion of sperm with unbalanced chromosomes resulting from the meiotic segregation, molecular cytogenetic analysis was done in sperm from a man heterozygous for a t (1,10) (p25 ; q32) reciprocal translocation (RT) using fluorescence in situ hybridization (FISH). Semen samples were obtained from a RT carrier and chromosomally normal men. Two color FISH using two DNA probes (CEP1, CEP10) and three color FISH using three DNA probes (CEP1, CEP10 and Tel 1q) was performed to analyze segregation patterns of chromosomes involved in the translocation. A total of 4664 sperm from a RT carrier and 3161 sperm from the normal men were scored. In two color FISH analysis, the frequency of sperm with alternate segregation or adjacent 1 segregation, adjacent 2 segregation and 3:1 segregation was 95.4%, 0.2%, 3.5% respectively in a RT carrier, while 99.5%, 0.1% and 0.3% respectively in the control men. In three color FISH analysis, the frequency of sperm with alternate segregation or adjacent 1 segregation, adjacent 2 segregation and 3:1 segregation was 88.4%, 1.0% and 5.8% respectively in a RT carrier, while 97.5%, 0.2% and 2.0% respectively in the control men. In the sperm of alternate segregation or adjacent 1 segregation in a RT carrier, 46.3% of them were balanced in the chromosomes involved in the translocation, while 42.1% of them were imbalanced. Overall, 48.9% of the sperm were chromosomally imbalanced in a RT carrier. These data indicate that at least about 50% sperm are produced by unbalanced segregation.

The parental origin of X chromosomes in 15 Mexican Turner patients with different karyotypes. *M. Lopez^{1,2}, N. Monroy¹, A. Cervantes¹, R. Guevara Yanez¹, D. Garcia-Cruz³, V. Mesa³, S. Canun⁴, G. Zafra⁵, J.C. Zenteno¹, S. Kofman¹.* 1) Genetica Hospital General Mexico, F Medicina UNAM, Mexico DF; 2) S Biologicos UAM-X, Mexico DF; 3) Genetica, CIBO IMSS, Guadalajara, Jal. Mexico; 4) Genetica Hospital Gea Gonzalez, Mexico DF; 5) Genetica Hospital Espanol, Mexico DF.

Several studies have shown that 40-60% of patients with Turner syndrome (TS) are monosomic for the X chromosome, the remaining cases have a structurally abnormal X or Y or are mosaics with a second cell line with a normal or abnormal sex chromosome. Experimental evidence has demonstrated that 70-80% of 45,X patients retain the maternal X chromosome, while X isochromosomes can be either paternally or maternally derived. We studied 15 TS patients, 10 were 45,X and the rest had a second cell line with abnormal X chromosomes: one pseudodicentric, one isochromosome, one large and one small ring, and one with a long arm deletion. In all cases exclusion of Y mosaicism was carried out by PCR amplification of five Y-specific sequences, including the centromeric region. Parental origin of X chromosomes was determined in genomic DNA by PCR using ten (CA/GT)_n X chromosome microsatellites (p22.3-q28) from panel 28 ABI-PRISM Linkage Mapping Set Ver 2.0 PE. PCR products were analyzed with ABI PRISM 310 genetic analyzer running GENESCAN 2.1 software. We were able to determine that the parental origin of the single X chromosome was maternal in 90% of the 45,X cases. The results were also used to corroborate breakpoints in the abnormal X chromosomes, particularly confirming the presence of the X inactivation center in the tiny ring chromosome [45,X/46,X,r(X)(p21q13)]. Of the structurally abnormal X chromosomes 3 were maternal [45,X/46,X,pseudodic(X)(p11.2); 45,X/46,Xr(X)(p22.3q28) and 45,X/46,X,del(X)(q23)], while the other two [45,X/46,X,i(Xq) and 45,X/46,X,r(X)(p21q13)] were paternal. These data are in agreement with previous reports. We discuss the timing and mechanism of error(s) leading to the formation of abnormal X chromosomes from maternal origin.

De novo interstitial deletion of 5p14 region in prenatal diagnosis. *Y.M. Kim¹, M.H. Lee¹, E.H. Cho¹, M.Y. Kim², J.Y. Han², H.M. Ryu².* 1) Laboratory of Medical Genetics, Samsung Cheil Hosp, Seoul, Korea; 2) Department of Obstetrics and Gynecology, Samsung Cheil Hospital & Women's Healthcare Center, School of Medicine, Sungkyunkwan University, Seoul, Korea.

There have been some G-band positive interstitial deletion cases without phenotypic effect such as 5p14, 11p12, 13q21 and 16q21. We report a de novo interstitial deletion of 5p14 region. A 34 year-old-woman with MCDA twin pregnancy was referred for amniocentesis due to high risk of Down syndrome at 17.3 wks of gestation. This pregnancy was done by ICSI after TESE. Karyotype of both fetuses revealed 46,XY,del(5p)(14) by conventional GTG-banding. In the parental study, they showed normal karyotypes. High resolution G-banding and reverse banding using amniocytes showed the interstitial deletion around 5p14. FISH showed positive signal at the 5p15.2 region and CGH (comparative genomic hybridization) confirmed the deletion of 5p14 region. However we couldn't find the exact breakpoint with all these results. The level II sonography (high resolution ultrasound) at 20 wks didn't show any special fetal physical abnormality. The parents declined further study with cordocentesis and wanted to keep their pregnancy. Further study is expected after birth.

Paracentric inversion of chromosome 12 [46,XX, inv (12)(q12;q24)] in a woman with recurrent fetal anomalies - Coincidental or causal? *D.S. Krishna Murthy, M.A. Redha, S.A. Gouda, L. Bastaki, S.A. Al-Awadi.* Cytogenetics Laboratory, Medical Genetics Center, Maternity Hospital, Sulaibikat, KUWAIT.

A normal healthy couple with history of multiple congenital anomalies and neonatal death in the offspring were investigated to exclude balanced chromosomal rearrangement. Routine chromosome analysis using G-banding technique confirmed paracentric inversion of chromosome 12 in the mother (46,XX, inv (12)(q12;q24)). Paracentric inversions (PAI) are relatively rare as compared to pericentric inversions (PEI), considered to be heteromorphic variants in the general population. There are very few reports of PAI involving chromosome 12. While PAI involving short segments are likely to be harmless, most of the abnormal zygotes resulting from unbalanced gametes produced by carriers of long PAI are likely to be so grossly abnormal that they would be lost very early, even before implantation. The majority of PAI carriers are apparently normal without any serious deleterious effect. PAI inversions are mostly ascertained among individuals with history of infertility couples with history of recurrent abortions and/or fetal anomalies, or parents of abnormal children. The risk of abnormal structural rearrangement in offspring of PAI carriers has been reported to be relatively small, as most of the affected zygotes from the unbalanced gametes are lethal or lost even before implantation. Couples with increased risk should be offered prenatal diagnosis. PAI of 12 has also been reported in human malignancies. A brief review of PAI involving chromosome 12 will be presented.

Three cases of unique duplication of the long arm of chromosome 2. *C.M. Lovell, A.S. Kulharya, W.H. Hoffman, D.B. Flannery.* Dept Pediatric Genetics, Medical Col Georgia, Augusta, GA.

Isolated duplication of the long arm of chromosome 2, particularly the proximal region is extremely rare. A distinctive phenotype has emerged for the distal partial trisomies for 2q. However, there is no consensus on the phenotype for proximal and middle segment duplications of 2q as very few cases of partial trisomy for these segments are reported. We present three patients with overlapping duplications of the proximal and middle segments of 2q.

The first patient is a 19 year old intellectually impaired male with indistinct speech, awkward gait, and hypotonia. He has hypogonadotropic hypogonadism. Karyotype is 46,XY,dup(2)(q23q33). The second patient is a 10 month old female with microcephaly, trigonocephaly, upslanting palpebral fissures, 2 posterior whorls, Brushfield spots, gross developmental delay, and hypertonia in lower extremities. Karyotype is 46,XX,dup(2)(q11.2q21.3). The third patient was a newborn with multiple congenital anomalies. The physical findings included overlapping fingers, cleft palate, horseshoe kidney, dextrocardia with right side aortic arch, seizures, and hypotonia. She died at 5 days of age from severe unremitting lactic acidosis which raised concerns about Finnish lethal neonatal metabolic syndrome [OMIM 603358], which has been mapped to the 2q32-37 region. Her karyotype was 46,XX,dup(2)(q14.2q31). Parental karyotype in all three cases were normal. The identity of the additional material was confirmed with FISH using WCP2.

Although several cases of duplication 2q have been reported earlier, the breakpoints seen in our patient have been not described previously. It appears that the larger partial trisomies for 2q are not tolerated as shown by the short life span of our third patient and also by the lack of reports of abnormal segregant products of reciprocal translocations with more proximal breakpoints in 2q. Our patients share some features described earlier for dup(2) patients such as urogenital anomalies. These patients further define the genotype phenotype correlation for the proximal half of the long arm of chromosome 2.

Prenatal detection and FISH characterization of a de novo unbalanced translocation between chromosomes Y and 12. X. Li, K. Wcislo, L. Bros, K. Hartley, C. Tse, J. Bergoffen. Genetics Department, Kaiser Permanente Northern California, San Jose, CA.

De novo unbalanced translocations are rare. We report a prenatal case with a de novo unbalanced translocation between the Yq and 12p. Patient was a 39 year-old G3P1SAB1 woman, and had amniocentesis for advanced maternal age. Conventional cytogenetic analysis showed a male karyotype with a derivative Y involving the Yq heterochromatin region, either a product of an unbalanced translocation of unknown origin or a benign variant. Clinical significance was uncertain. Genetic counseling was further complicated by a normal male karyotype from a previous pregnancy with the same partner two years ago and normal karyotypes from both parents. The Y chromosome from the father appeared to be the same as in the first fetus. Non-paternity was strongly denied. No growth anomalies were seen by ultrasound at 18 weeks. Fluorescence in situ hybridization (FISH) using DNA probes specific for the XpYp and XqYq telomeric (pseudoautosomal) regions (from Cytocell, Ltd) revealed normal hybridization patterns for the XpYp and Xq but a deletion for the Yq (DXS7059-). A small portion of the terminal material on the Yq was negative for a whole chromosome Y paint probe (wcpY-) but positive for a 12p telomeric probe (Tel12p+) when screened with an all telomeric probe panel (Cytocell, Ltd.). There was no evidence that other chromosomes were involved in this rearrangement. Therefore, the derivative Y most likely results from a de novo unbalanced translocation between the Yq and 12p. This results in partial monosomy for Yq12 and partial trisomy for 12p13. Individuals with partial trisomy 12p are reported to have mental retardation, hypotonia, and minor dysmorphic features but no major malformations. Follow-up cytogenetic studies on a repeat amniocentesis sample as requested by the patient demonstrated the same abnormality and therefore ruled out a lab error. The pregnancy was terminated after the results were presented to the patient. We demonstrate here that the FISH technology allows us to distinguish a pathological chromosomal rearrangement from a possible benign Yq variant.

Screening for subtle structural anomalies by use of subtelomere specific FISH probe set. *S.M. Jalal¹, A. Harwood¹, M. Anderson¹, C. Lorentz¹, M. Law¹, N. Lindor¹, P. Karnes¹, A. Kulharya², G. Sekhon³, V. Michels¹.* 1) Mayo Clinic, Rochester, MN; 2) Medical School, Augusta, GA; 3) University of Wisconsin, Madison, WI.

A complete set of human telomere specific probes were developed in 1996 and are now commercially available. Due to a high concentration of genes in telomeric regions, these probes are being used with intense interest to investigate individuals with nonspecific mental retardation (MR), couples with multiple miscarriages (MM) and children with nonspecific mild dysmorphic features. We present our findings involving such individuals and children with mild to severe dysmorphic features that were karyotypically normal. The study included 22 individuals with MM and 27 individuals with a range of dysmorphism and MR. The probe set (43 probes) included all telomeres except the p-arms of acrocentric chromosomes. We were careful in the interpretation as some of the probes produce weak signals on certain chromosomes due to cross hybridizations. Whenever an anomaly or a polymorphic variant was discovered it was confirmed by arm specific probes from another company. We discovered two terminal deletions involving 22q and 10q in two children with moderate to severe dysmorphism, and a complex rearrangement involving 3pter and 3qter in two cases. A euchromatic subtle deletion (that includes D2S2986) variant of 2q was observed in four individuals including a child with severe dysmorphism and a phenotypically normal father. The 2q probe from one source was deleted in all four while the probe of the other company was not deleted in any, leading to the discovery of the normal variant. In addition, these subtelomeric probes were invaluable as markers in cases of r dup(14), r(21), inv dup(6) and mosaic balanced t(3;12). We conclude that with significantly improved quality, the commercially available subtelomeric probes are now effective in the detection of subtle anomalies in a wide range of situations.

Chromosomal Abnormalities in Cell Cultures from the Baltimore Longitudinal Study: Correlations with Donor Age. *J.C. Leonard, A. MacMillan, M.A. Wineburg, P.E. Fuller, R.T. Johnson.* Coriell Cell Repositories, Coriell Inst Medical Research, Camden, NJ.

The Baltimore Longitudinal Study is a continuing survey of the health of volunteers ranging in age from 20s to 90s conducted by the Gerontological Research Center. As part of this study, skin fibroblast cultures have been banked in the NIA Aging Cell Repository and cytogenetic analysis is part of the standard characterization of all cultures in the NIA Repository. Studies of chromosomal aberrations that occur spontaneously in cell cultures have suggested that genomic damage is a factor in the aging process. A review of the 279 routine quality control assays of GRC cultures performed since 1995 considers a larger number of analyses than all of the previous studies combined. Our results confirm that both structural and numerical chromosome abnormalities are commonly observed in low passage fibroblast cultures from adult donors and high levels of these coincide with poor growth and the onset of in vitro senescence. Furthermore, a trend toward increased loss of chromosomes is seen among cultures from donors older than 80, although no age association was seen for other aberration types. As would be expected from previous data, loss of sex chromosomes is the most common cause of mosaic aneuploidy. The nonrandom distribution of structural aberrations suggests some loci are hotspots for chromosomal exchanges. Some but not all of these hotspots are in chromosome bands that are recognized as common fragile sites. Expression of these hotspots is not related to donor age, but more data is needed to determine if they predominate among the aberrations seen with the onset of in vitro senescence.

Sexual ambiguity in a child with a karyotype 46,XY,del(2)(q32.1-q35) and phenotype resembling the Smith-Lemli-Opitz syndrome. *J. Lavoie¹, F. Gros-Louis¹, M. Bronsard¹, R. Laframboise², B. Fouquette³, R. Drouin¹.* 1) Unite Recherche Genetique Humaine et moleculaire, Universite Laval, CHUQ, Hopital St-Francois d'Assise, Quebec, PQ., Canada; 2) Pediatrics Department, Medical Genetics, CHUL, CHUQ; 3) Division of pathology, Hopital St-Francois d'Assise, CHUQ.

We report a 3-year-old boy with a clinical phenotype that was at birth compatible with a Smith-Lemli-Opitz syndrome (SLOS). Clinical findings of the new born included craniofacial, genitourinary and intestinal malformations, and later on developmental delay. Despite normal G-banded karyotype on amniocytes, high-resolution banded chromosome study that was carried out on peripheral blood sample permitted the identification of a de novo interstitial deletion of the long arm of the chromosome 2. Molecular analysis using microsatellite markers indicated that the deletion originated from the maternal chromosome and was located between bands 2q32.1 and 2q35. This case is one of the rare cases of deletion 2q presenting abnormal external genital organs. The sexual ambiguity seen in this patient suggest a gene involved in sex determination in this chromosomal region. Moreover, one locus for cleft palate has been recently located to band 2q32. Since our patient does not present this malformation, it therefore contributes to define more precisely the locus to band 2q32.1. Finally, this case denotes the importance of high-resolution chromosome analysis during the course of prenatal and postnatal cytogenetic investigations, in order to ensure an adequate genetic counseling.

Variable outcomes in 5 cases of mosaic trisomy 16. *P.M. Hohler¹, K. Neiswanger², L. Thomas¹, P. Mowery-Rushton¹, E. McPherson^{1,2}, A. Hogge^{1,2}, U. Surti^{1,2}.* 1) Dept of Genetics, Magee Womens Hospital, Pittsburgh, PA; 2) University of Pittsburgh, Pittsburgh, PA.

We report 5 cases of mosaic trisomy 16. 1) A 41yo, with 100% 47,XY,+16 by CVS and 100% 46,XY by AF, delivered an IUGR, but otherwise normal male infant at 33wks. At birth, a normal karyotype was seen in blood and cord samples, while the placenta was mosaic for trisomy 16. The disomic cells were biparental. The trisomy showed CPM, and was maternal and meiotic in origin. 2) A 25yo presented with IUGR by US and 12% 47,XY,+16 by AF. At 36wks, she delivered a male infant with IUGR, plagiocephaly, and VSD. The blood karyotype was normal. DNA analysis revealed a maternal, meiotic non-disjunction, with UPD. 3) A 31yo with elevated hCG, 7.66 MoM, a two-vessel cord by US, and level II mosaicism for 47,XY,+16 by AF, delivered at 37wks an IUGR male infant with multiple skeletal anomalies and dextrocardia. The cord blood karyotype was normal, while the cord tissue was 32% trisomy 16. 4) A 41yo showed 47,XX,+16 by CVS, an elevated hCG, 3.2 MoM, and 60% trisomy 16 by AF. US revealed normal growth with a heart defect. At termination, 46,XX karyotypes were seen in brain, tongue, spleen, and skin, with mosaic trisomy 16 in liver (80%), cord (75%), lung (40%), and muscle (20%), using cytogenetics and FISH. 5) A 33yo presented with elevated hCG, 16.5 MoM. AF revealed level II mosaicism for 47,XY,+16. At 32wks she delivered a male infant with heart defects, who died on day 2. No autopsy was performed. The cord blood karyotype was normal, while trisomy 16 was seen in multiple placental and cord samples by FISH and cytogenetics. The complications in these 5 pregnancies included preeclampsia, IUGR, fetal anomalies and death, with no predictable pattern. In case 4, trisomy 16 showed a variable distribution among tissues. Cases 3 and 5 had level II mosaicism for trisomy 16 by AF. The majority of level II mosaicisms are considered to be pseudomosaicism. However, in both of these cases the trisomic cell line was confirmed upon further study. Our understanding of mosaic trisomy 16 will be greatly enhanced by the use of FISH, in conjunction with other newly evolving cytogenetic and molecular techniques.

Clinical and cytogenetic findings in an infant with rec(8)dup(8p) and mosaic trisomy 8. *B.N. Hay¹, S.A.*

Schonberg², F. Lacbawan³. 1) National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 2) Cytogenetics, American Medical Laboratories, Inc., Chantilly, VA; 3) Department of Medical Genetics, Children's National Medical Center, Washington, DC.

We describe a patient who presented at 6 weeks of age with macrocephaly, large anterior fontanelle, hypertelorism, large ears, and broad digits. Karyotype analysis revealed the mosaicism 46,XY,add(8)(q24.3)[15]/47,XY,+8,add(8)(q24.3)[5]. The add(8) banding pattern was most consistent with the interpretation rec(8)dup(8p). This was confirmed by parental chromosome analysis, which revealed a maternal balanced pericentric inversion of chromosome 8 (p21.1q24.3) and defined the probands karyotype as rec(8)dup(8p)inv(8)(p21.1q24.3). In combination with the mosaic trisomy 8, 8p is duplicated in 75% of cells and triplicated in 25% of cells. While both distal duplication 8p and mosaic trisomy 8 have a well-defined phenotype, neither this combination of findings, nor the chromosomal dose abnormality have been previously described. Subsequent to diagnosis, a brain MRI revealed a hypoplastic corpus callosum with absent rostrum plus a diminutive optic chiasm and optic tracts. In return visits, the infant has demonstrated hypotonia, developmental delay, and a decrease in growth rate. We discuss mechanisms leading from the maternal inv(8) to both rec(8) and a presumed chromosome 8 meiotic non-disjunction and compare clinical findings with prior reports of both dup(8p) and trisomy 8 mosaicism.

Neocentromere in a ring-shaped chromosome 1. *R.R. Higgins¹, E. Wright², S. Baldinger³, E. Tschider¹, J. Ahmad¹, S. Schwartz⁴, C.A. Curtis¹.* 1) Allina Cytogenetics Lab, Abbott Northwestern Hosp, Minneapolis, MN; 2) Haislet, Wavrin, Wright, Lehrman & Associates, Edina, MN; 3) Minnesota Perinatal Physicians, Abbott Northwestern Hosp, Minneapolis, MN; 4) Case Western Reserve University, Cleveland, OH.

We describe a neocentromere in a ring-shaped chromosome involving the long arm of chromosome 1. A G₅P₂AB₂ 39-year-old woman was referred for amniocentesis at 15 weeks of pregnancy for advanced maternal age. Cytogenetic analysis of 20 colonies from 5 coverslips showed a male karyotype with an interstitial deletion in the long arm of a chromosome 1 involving the G-band breakpoints q23 and q32, plus a ring-shaped marker chromosome derived from chromosome 1 involving the G-band region 1q23-q32 [47,XY,del(1)(q23q32),+r(1)(q23q32)]. The abnormalities appeared to be de novo since chromosomal analyses of the patient and her spouse were normal. The ring-shaped chromosome was diagnosed as chromosome 1 chromatin by fluorescence in situ hybridization (FISH) using a "painting" molecular probe specific for chromosome 1. The ring chromosome showed no evidence of a centromere by CBL-staining nor when studied by FISH using a pan alpha-satellite probe and a chromosome 1 alpha-satellite probe. Three of the twenty metaphases studied were missing the ring chromosome, however no colonies having all cells missing the ring chromosome were found. The three metaphases missing the ring chromosome may have been cultural artifacts and not representing true mosaicism. In eleven of the rings studied, eight were approximately the size of the short arm of a chromosome 20 with the 1q23-q32 banding, two rings were half that size with less banding, and one ring was as large as an entire chromosome 20 with additional banding. Thus, the ring chromosome was demonstrating "dynamic mosaicism". An ultrasound analysis at 18 weeks was normal, but the pregnancy was terminated due to the risk of a ring syndrome. The ring chromosome appears to have a neocentromere without alpha-satellite DNA. The ring chromosome is being studied for the presence of CENP-B, -C, and -E.

Paternally transmitted recurrent true-hermaphroditism associated with SRY mosaicism. C. Oddoux¹, H. Yee¹, V. Clarke¹, C.M. Clayton¹, K. McElreavey², M. MacGillivray³, H. Ostrer¹. 1) Div Human Genetics, New York Univ Schl Med, New York, NY; 2) Institute Pasteur, Paris, France; 3) Div Ped Endo, Buffalo Children's Hosp, Buffalo, NY.

In 1987 some of us described a family with coexisting XX chromosomal maleness and true hermaphroditism with 4 affected individuals spanning two generations and displaying a paternal inheritance pattern. Karyotyping of 3 affecteds and their parents revealed normal chromosomes (all affecteds 46,XX). We used DNA probes flanking the testis determining region in Southern analyses of peripheral leukocyte DNA of 2 of the XX males, their normal sister and their father to rule out a Y-to-X or a Y-autosomal translocation of this portion of the Y chromosome. Similarly probes to the pseudoautosomal region were used to rule out the presence of a Y chromosomal inversion polymorphism which could lead to recurrent translocation. PCR analysis with primers to the SRY gene failed to demonstrate its presence. After attaining puberty, gynecomastia was noted in each of these individuals and gonadectomies were performed. Pathological examination of the gonadal tissue indicated that the individuals were in fact true hermaphrodites. Here, we present molecular analyses performed directly on the paraffin-embedded gonadal tissue. Immunohistochemistry using a specific anti-SRY antibody revealed high levels of SRY expression in testicular tubules, some expression in oocytes, and no expression in connective tissue or blood vessels. PCR analysis of gonadal DNA confirmed the presence of the SRY gene but at levels lower than observed in normal males, consistent with the histochemical mosaicism. Individuals mosaic for cells with and without Y chromosomes or Y-chromosomal fragments containing the SRY gene have previously been reported in *sporadic* cases of true hermaphroditism. Suggested mechanisms have included embryonic correction of Klinefelter syndrome or triploid fetuses, or chimerism resulting from the fusion of an independently fertilized oocyte and polar body. These mechanisms are not consistent with recurrent paternal transmission of SRY gene mosaicism and this family provides evidence for existence of a novel mechanism.

Wolf-Hirschhorn syndrome: Correlation between cytogenetics, FISH, and severity of disease. *A.M. Meloni¹, R.R. Shepard¹, A. Battaglia^{2,4}, T.J. Wright³, J.C. Carey⁴.* 1) Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; 2) Stella Maris Scientific Research Institute, University of Pisa, Calambrone, Pisa, Italy; 3) Department of Human Genetics, University of Utah, Salt Lake City, UT; 4) Division of Medical Genetics, Department of Pediatrics, University of Utah, Salt Lake City, UT.

Wolf-Hirschhorn syndrome (WHS) is a multiple malformation syndrome characterized by the deletion of the distal short arm of one chromosome 4. Clinical features include a prominent forehead with widely spaced eyes, microcephaly, distinct mouth, as well as mental and growth retardation. Nineteen patients were included in the study (10 cases from the USA and nine cases from Italy). The study was designed to characterize the size of the deletion by cytogenetic and FISH methods and to correlate the results with the severity of the disease. Standard cytogenetics was performed following established methods. FISH studies were carried out using either a commercially available probe (four cases) or a panel of 8 markers known to map within and in proximity to the WHS critical region. Fifteen of the 19 patients analyzed by standard cytogenetics had a cytogenetically visible deletion of the short arm of chromosome 4: eight cases had a small deletion with the breakpoint at 4p16, whereas a larger deletion with the breakpoint at 4p15.3 was observed in seven cases. Four patients had an apparently normal karyotype. FISH was performed in 13 of the 19 cases included in the study. Three of the four cases with an apparently normal karyotype were found to be deleted by FISH. The study definitely showed that the four cases with an apparently normal karyotype had clinical features varying from mild to very severe. In addition, severe clinical features were observed in patients with small deletions, and mild clinical features were seen in patients with large deletions. The results of this study suggest that the size of the deletion on chromosome 4 is not solely responsible for the severity of the clinical features. Therefore, in addition to the deletion of the WHS critical region, a modifying factor may be contributing to the clinical presentation of the disease.

Full trisomy 8p identified as inv dup (8p) without deletion of subtelomere. *P.N. Mowrey¹, J.H. Tepperberg¹, I.K. Gadi¹, K.K. Phillips¹, L. Wisniewski¹, P.R. Papenhausen¹, K. Paulyson-Nunez².* 1) Diagnostic Genetics, Laboratory Corporation of Am., Research Triangle Pk, NC; 2) Dept. OB/GYN, Duke U Med Cnt, Durham, NC.

We report a 21 y.o. Hispanic female, G1P0, referred for ultrasound dating. Findings include a 20 week gestation with a 2 vessel cord, and a prominent stomach. A repeat scan at 24 weeks further revealed asymmetric echogenicity of kidneys, colpocephaly, and possible agenesis of the corpus callosum. A heart defect was suspected. The family history was unremarkable. MSAFP was normal. Amniocentesis was performed and chromosome analysis showed a GTW banding pattern consistent with an apparent atypical short arm inverted duplication of chromosome 8 in all colonies. The dup (8) appeared to contain 2 copies of the 8p11.2 to 8p23.2 bands with the telomere region 8p23.3 in between. FISH analysis, using a whole chromosome 8 "painting" probe, revealed complete DNA hybridization with the entire chromosome 8, confirming that the extra material was of chromosome 8 origin and supporting the banding observation. Additional FISH testing, using an 8p subtelomere probe, supported the presence of a telomeric region just proximal to the dup 8p material. Alphoid FISH studies showed a single pericentromeric hybridization signal. These results support an inverted duplication of chromosome 8 short arm including the entire 8p23 region, which is generally deleted in inv dup (8p) cases. This case also demonstrated an unusual banding pattern beyond the distal end of the duplicated segment that is apparently 8 paint positive but negative for the telomere and centromere probes. Additional molecular testing with BACs will be performed to further define this region. Parental chromosome analyses are pending. This case of whole short arm duplication will be contrasted with cases in the literature that show loss of the telomere.

Prenatal diagnosis of "7q syndrome". *G.S. Kupchik¹, M. Velinov^{1,3}, D. Rosa¹, M. Silverman², T. Shklovskaya², S.M. Kleyman², M.J. Macera², R.S. Verma².* 1) Division of Genetics, Maimonides Medical Ctr, Brooklyn, NY; 2) Wyckoff Heights Medical Center, Brooklyn, NY-Weil Medical College of Cornell University, NY,NY; 3) NYS Institute for Basic Research, Staten Island, NY.

A 25 year-old Caucasian woman of Jewish ancestry was referred for amniocentesis at 32 weeks of gestation due to significant IUGR of the fetus. Cytogenetic findings for the fetus with G-banding revealed an abnormal karyotype, 46,XY,del(7)(q32). The pregnancy was continued and the child was born at 34 weeks gestation after otherwise unremarkable pregnancy. The birth weight was 920g demonstrating severe IUGR. The newborn presented with single umbilical artery, micrognathia, low set ears, and prominent nose. In addition, he had hypoplastic nipples, mild hand contractures, hypospadias, undescended testes, and enlarged distance between first and second toes. The echocardiogram and renal sonogram were unremarkable. A family history was obtained following birth and was significant for the couple's 4.5 year old son with developmental delay and chronic lung disease, and 3 year old son with macrocephaly and hypotonia. The cytogenetic analysis of the newborn's blood confirmed the initial diagnosis. FISH analysis with whole chromosome painting probe and telomere probe for chromosome 7 (Vysis) were applied. telomeric signals were only detected over the normal chromosome 7. The karyotype was thus reported as 46.XY,del(7)(q32).ish(wcp7+,Telyvision7-). The parents declined the recommendation that they undergo chromosome analysis to evaluate the possibility of a familial translocation. Since 1968, over 60 cases with various 7q deletions have been reported. Chromosomal breaks have been reported at 7q32, 7q33, 7q34 and 7q35. Nevertheless, the most frequent breakpoints were at 7q32, resulting in growth and mental retardation, cleft lip/palate, as well as lack of significant neurological findings. In conclusion, the severity of the clinical manifestation in this case does not appear to be proportionately related to the size of the missing chromosomal segment. In our view this patient presented with phenotype on the milder end of the previously reported cases of 7q deletion with comparable size.

Double Trisomy. *S. Li, S. Hased, J.J. Mulvihill, A.K. Nair.* Department of Pediatrics, The University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Double trisomies are extremely rare, and most of them involve the sex chromosomes (XXX, XXY or XYY) combined with either trisomy 13, 18 or 21. We present a case of a baby girl with non-mosaic double trisomy 48,XXX,+18, detected after birth. Fluorescence in situ hybridization (FISH) analysis with DNA probes specific for the X and 18 chromosomes was utilized to confirm routine cytogenetic studies in both metaphase and interphase cells. We have also reviewed the literature on previously reported cases of double trisomy and discussed implications of double trisomy.

Cytogenetic and molecular studies of a de novo mosaic trisomy 12p. *K. Krabchi¹, J. Lavoie¹, R. Laframboise², M. Bronsard¹, R. Drouin¹.* 1) Dept Medical Biol, Laval Univ, Quebec City, Quebec, Canada; 2) Pediatrics Dept, Medical Genetics, CHUL, CHUQ, Quebec City, Quebec, Canada.

The peripheral blood sample of a 12-year old boy presenting a phenotype resembling to a Fragile X syndrome has been addressed for both cytogenetic and molecular studies. Based upon GTG banding, the constitutional karyotype of the proband revealed the presence of two cellular clones: mos46,XY,22p+[25]/46,XY[75]. Successive FISH analyses using different coatasomes allowed us to identify the translocated fragment on the chromosome 22 as being from chromosome 12. The subtelomeric probe specific for chromosome 12p was hybridized to identify the materiel translocated to chromosome 22p. This one has precisely defined its origin. The breakpoints have been mapped using high-resolution GTG and RBG banding. Ag-NOR technique, specific for the argentaaffin region bearing the nucleolar organizer regions, showed the integrity of this region on the derivative chromosome. This chromosome is, therefore, constituted of the intact long arm of chromosome 22, the active centromere, the achromic Giemsa zone and the NOR, all directly associated to the short arm segment of chromosome 12. In this rare event, the breakpoints occurred within the band 22p13. In order to continue our investigation, and considering the data from the literature concerning the Pallister-Killian syndrome (PKS), we performed a cytogenetic analysis of fibroblasts obtained from a skin biopsy. Usually, PKS patients have a tissue-specific tetrasomy 12p, mostly mosaic in the lymphocytes, but always homogenous in the fibroblasts. In our case, the translocation 12;22 is found in almost 100% of the fibroblasts. This unusual case, by its similarity with PKS and the presence of active NORs on the rearranged chromosome 22 requires more investigations, particularly determine the parental origin of the proband's chromosome abnormality.

Prenatal Diagnosis of a De novo Ring Chromosome 11. *A.N. Mohamed¹, S.A. Ebrahim¹, R. Aatre², M.I. Evans².* 1) Dept of Pathology/Cytogenetics,; 2) Dept of Obstetrics and Gynecology/Division of Reproductive Genetics, Wayne State university, Detroit, MI.

Ring chromosomes are uncommon findings in prenatal diagnosis. Growth retardation is the most significant manifestation in particular among patients with ring of larger chromosomes. A 30-year-old gravida 1, para 0 white woman was referred for genetic counseling because of maternal anxiety. An amniocentesis was performed at 18 week 6 day of pregnancy. Cytogenetic analysis revealed an abnormal mosaic female chromosome complement;46,XX,r(11)(p15q25)[14]/ 45,XX,-11[7]. The ring 11 showed no detectable loss of chromosomal material at 450-band level. Chromosomal analysis of both parents' blood showed normal karyotypes. A targeted ultrasound evaluation at time of consultation revealed no significant abnormality. The parents were counseled regarding the possible developmental abnormalities of the fetus based on the available information in the literature. Subsequently, the parents decided to terminate the pregnancy. Chromosome analysis of the fetal skin biopsy confirmed the same abnormality seen in the amniotic fluid culture. The autopsy revealed an immature female fetus with dysmorphic craniofacial features including brachycephaly, low-set ears, and hypertelorism, bicornuate uterus, and calcifications in renal tubules. The abnormal phenotypes could be a consequence of the ring instability, submicroscopic deletion and/or alteration at the site of fusion.

Hypomethylation, heterochromatin decondensation, and rearrangements targeted to the pericentromeric regions of chromosomes 1 and 16 in cell lines from ICF syndrome patients and in chorionic villus cultures from normal fetuses.

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The ICF syndrome (immunodeficiency, centromeric region instability, facial anomalies) is a unique DNA methyltransferase deficiency disease with anomalies in the juxtacentromeric regions (qh) of chromosomes 1 and 16 (Chr1 and Chr16) in mitogen-stimulated lymphocytes. These aberrations are qh decondensation and multiradial chromosomes with up to 12 arms, isochromosomes, translocations, and whole-arm deletions. Our previous studies of ICF LCLs and 1qh and 16qh hypomethylation in cancer suggest that multiradials may give rise to more stable pericentromeric rearrangements. We report that high levels of spontaneous pericentromeric abnormalities were targeted to Chr1 and Chr16 in late-passage chorionic villus (CV) cells from randomly chosen fetal samples as well as in three ICF lymphoblastoid cell lines (LCLs) but not in normal LCLs and fibroblast cell strains. Decondensation of 1qh and 16qh was more frequent than pericentromeric rearrangements. The three ICF LCLs had different frequencies of these anomalies. The more of this decondensation that was seen in 1qh and 16qh in a given ICF LCL, the higher the level of 1qh and 16qh rearrangements in that LCL. A similar relationship suggesting that heterochromatin decondensation predisposed to rearrangements in cis was observed for late-passage CV cells. However, more pericentromeric chromatin breaks and chromosome breaks and fewer multiradials were seen in Chr1 and Chr16 in CV cells than in the ICF LCLs. Like ICF cells, these CV cell samples were hypomethylated in the main satellite DNA in this region (satellite 2). Also, treatment of a normal pro-B human cell line induced high frequencies of decondensation of 1qh and 16qh and high levels of demethylation in this heterochromatin. Our results are consistent with the hypothesis that DNA hypomethylation predisposes to heterochromatin decondensation which, in turn, can lead to rearrangements in cis. (Supported by PHS Grant CA81506.).

Cigarette smoking and aneuploidy in human sperm. *Q. Shi^{1,2}, E. Ko², L. Barclay², T. Hoang¹, A. Rademaker³, R. Martin^{1,2}.* 1) Department of Medical Genetics, The University of Calgary, Calgary, Canada; 2) Genetics Department, Alberta Children's Hospital, Calgary, Alberta, Canada; 3) Cancer Center Biometry Section, Northwestern University, Chicago, IL.

There have been concerns that smoking may increase the aneuploidy frequency in human sperm, because cigarette smoke contains chemicals which are capable of inducing aneuploidy in experimental systems and these chemicals have been shown to reach the male reproductive system. Recently, one study found no increased disomic sperm in smokers and three others examined several categories of disomy and suggested an association between smoking and only a single category of disomy. In these three studies, however, the authors could not rule out combined lifestyle factors as contributory as smokers also consumed more alcohol and/or caffeine than nonsmokers. In our study, semen samples were collected from 31 men with similar demographic characteristics and lifestyle factors except for cigarette smoking. None of the men drank alcohol. These men were divided into three groups: nonsmokers (10 men), light smokers (<20 cigarettes/day, 11 men) and heavy smokers ([≥] 20 cigarettes/day, 10 men). There were no significant differences in semen parameters or age across groups. Two multicolor fluorescence in situ hybridizations (FISH) were performed: two-color FISH for chromosomes 13 and 21, and three-color FISH for the sex chromosomes using chromosome 1 as an internal autosomal control for diploidy and lack of hybridization. Approximately 10,000 sperm/chromosome probe/donor were analyzed. The mean hybridization efficiency was 99.8%. The frequencies of X-bearing and Y-bearing sperm were not significantly different from the expected 50% for each of the three groups. The frequencies of disomy 13 were significantly higher in light and heavy smokers than in nonsmokers. A significant decrease was found in diploidy in X/Y/1 hybridization in both light and heavy smokers, and in XY disomy in light smokers. Thus there were no consistent increases in smokers compared to controls. Based on this study and literature, we can conclude that cigarette smoking may increase the risk of aneuploidy only for certain chromosomes.

Clinical and Cytogenetic Findings from Saudi Arabia: A Report of 6 Abnormal Cases. *S.A. Tashkandi, B. Al Muzaini, R.K. Graham, H. Al Nafae, I. Abdulkarym.* Riyadh Armed Forces Hospital, Riyadh, Saudi Arabia.

This is a presentation of six abnormal cases from the cytogenetics lab at RAFH with the aim of correlating the clinical and cytogenetic findings with the published literature. The first case is a family study of 8 members. Chromosomal analysis for the father revealed: 46,XY,t(2;18)(p25.3;p11.2). The maternal karyotype is normal. They have 6 children with the following karyotypes: normal female, carrier female, two females with the karyotype 46,XX,der(18)t(2;18)(p25.3;q11.2)pat and a male and a female with the karyotype 46,XY,der(2)t(2;18)(p25.3;q11.2)pat and 46,XX,der(2)t(2;18)(p25.3;q11.2)pat. The second case is a family study of 6 members. Chromosomal analysis on the mother revealed: 46,XX,t(12;18)(p13.1;q21.3). The paternal karyotype is normal. Out of 4 children, one is normal and three died in early infancy with a phenotype suggestive of trisomy 18. All three contained the following karyotype: 46,XX,der(18)t(12;18)(p13.1;q21.3)mat or 46,XY,der(18)t(12;18)(p13.1;q21.3)mat. The third case, a 20 year old female, presented with primary amenorrhea. Cytogenetic analysis disclosed a male karyotype with a balanced reciprocal translocation: 46,XY,t(6;7)(q21;q32). The fourth case, a 1 year old female, presented with dysmorphic features including cleft palate. Chromosomal analysis revealed two different cell lines resulting in a mosaic trisomy 13 state in 16% of the cells: 45,XX,rob(13;14)[84%]/46,XX,rob(13;14),+13[16%]. The last two cases revealed a similar cytogenetic finding however with different phenotype. One case is of a 1 year old male with a phenotype suggestive of Prader-Willi Syndrome. Chromosomal analysis revealed a duplication of band q11q13 on chromosome 15: 46,XY,dup(15)(q11q13). FISH analysis using the ONCOR Prader-Willi microdeletion probe SNRPN confirmed this abnormality. Conversely, a 36 year old male with a normal phenotype was referred for parental karyotyping after amniocentesis performed on his wife (in another hospital) showed a variant chromosome 15. The maternal karyotype was normal. The paternal karyotype was: 46,XY,dup(15)(q12q13).

Sperm Aneuploidy in Pesticide Appliers. *J.L. Smith*¹, *V.F. Garry*², *A.W. Rademaker*³, *R.H. Martin*^{1,4}. 1) Dept. of Medical Genetics, Faculty of Medicine, University of Calgary, Calgary, AB, Canada; 2) University of Minnesota Laboratory of Environmental Medicine and Pathology, Minneapolis, MN; 3) Cancer Center Biometry Section, Northwestern University, Chicago, IL; 4) Dept. of Genetics, Alberta Children's Hospital, Calgary, AB, Canada.

Male pesticide appliers in the Minnesota Red River Valley have significant decreases in fertility and their offspring have significant increases in birth defects when compared to the general population of the same region. Agricultural chemicals such as insecticides and fungicides utilized in this area include known aneugens. As aneuploid sperm can contribute to decreased fertility and an increased frequency of birth anomalies, this study is investigating the association between male pesticide exposure and aneuploidy in human sperm. Of 40 men, half were actively working as pesticide applicators. These 20 men had the highest exposure of 90 pesticide appliers studied. The 20 controls (matched for age and smoking) have had either low or no exposures previously, but had not been exposed to the chemicals in the past 3 years. Chromosomes 13, 21, X and Y were analyzed using multi-colour FISH (Fluorescence in situ Hybridization). A minimum of 20,000 sperm was scored for each donor - 10,000 for the 13, 21 chromosome pair and 10,000 for the XY pair. An autosome labelled with a third fluorescent colour was used with the sex chromosome pair to distinguish between XY diploidy and disomy. Analysis was performed "blindly" on coded slides. Aneuploidy frequencies in pesticide appliers vs. controls were: 0.44% vs. 0.36% for disomy 13; 0.44% vs. 0.52% for disomy 21; 0.05% vs. 0.05% for XX; 0.05% vs. 0.05% for XY; and 0.5% vs. 0.05% for YY. Diploidy in appliers vs. controls was 1.15% vs. 0.75% for chromosomes 13 and 21, and 0.23% vs. 0.05% for XY. Results thus far (with 75% of the data collected) demonstrated no significant increase in sperm aneuploidy, diploidy, or sex ratio with general pesticide exposure. However, after completion, further analysis will be carried out to compare men exposed to specific types of pesticides and also include data on lifestyle factors in combination with pesticide exposure.

Fusion of 9 beta satellite and telomere (TTAGGG)_n sequences results in a jumping translocation. *K.S. Reddy¹, T. Murphy²*. 1) Dept Cytogenetics, Quest Diagnostics, Inc, San Juan Capistrano, CA; 2) Memorial Hospital of Burlington County, Mount Holly, NJ 08060.

A newborn was found to have an isochromosome for the short arm of chromosome 9, i(9p) and a jumping translocation of the whole long arm. In 94.4% metaphases, 9q was fused to the telomere of chromosome 19p and, in 5.6% of metaphases, 9q was fused to the telomere of chromosome 8p. The net result was trisomy for the short arm of chromosome 9. With the pan telomere probe, FISH investigations found an interstitial telomere on the der(19) and der(8). The 9 beta and classical satellite probes gave a signal only on the long arm of chromosome 9 involved in the jumping translocation. The 9 alpha satellite probe hybridized to i(9p) and not to the other derivative chromosomes. A combination of chromosome 9 (red) and chromosome 19 (green) paint probes used to rapidly screen metaphases for the jumping translocation found 88 metaphases had a der(19)t(9;19) and 4 metaphases had a der(8)t(8;9). For the first time, the junction of a jumping translocation has been shown to involve the telomere sequence (TTAGGG)_n and beta satellite sequences by FISH. An intriguing model for jumping translocation will be proposed.

No significant role for a placental diploid cell line in ongoing trisomy 13 or 18 pregnancies. *G.H. Schuring-Blom¹, K. Boer², N.J. Leschot¹*. 1) Dept. of Clinical Genetics, Academic Medical Center, Amsterdam, The Netherlands; 2) Dept. of Obstetrics and Gynecology, Academic Medical Center, Amsterdam, The Netherlands.

In cytogenetic surveys of spontaneous abortions, trisomies for almost every chromosome have been described. A striking difference is observed in intrauterine survival: most trisomies are lethal, whereas a number of them lead to liveborn deliveries. For trisomy 21 20-35% survive to term, while less than 5% of the trisomy 18 cases and 2.5% of trisomy 13 cases are liveborn. To explain these differences it has been suggested that possibly only cases of trisomy 13 or 18, supported by a diploid cell line in the cytotrophoblast, might survive prenatally (trisomic zygote rescue). So far a limited number of trisomy 13 or 18 placentas (pregnancies between 14-40 weeks) has been investigated, partly using conventional cytogenetic methods, partly using fluorescence *in situ* hybridization (FISH). Only one or few sites were analysed, most of them showing mosaicism, some with high levels of diploidy, supporting the concept of trisomic zygote rescue. We set up a study to determine whether a diploid cell line is always present in the placenta. We investigated viable cases of trisomy 13 (n=8) or 18 (n=6) of live newborns (n=5) and terminated pregnancies (n=9). Gestational ages were between 15-37 weeks, comparable with earlier studies, mean maternal age was 34.7 years. Five random biopsies were taken from each placenta and FISH on cytotrophoblast cells (n=100) was performed with chromosome 13 or 18-specific probes. In neither the trisomy 13 nor the trisomy 18 placentas fully diploid sites were observed. In one trisomy 13 placenta low levels of diploid cells (13-20%) were seen at all sites. In two cases of trisomy 18 somewhat higher levels were observed (respectively 17-33% and 12-45%). In all other cases full trisomy was seen at all sites. We have found that the presence of a diploid cell line in the outer layer of the placenta (cytotrophoblast cells) is not a determining factor for survival in these 14 cases of trisomy 13 or 18, conflicting with earlier studies. Although trisomic zygote rescue may exist, other, as yet unknown factors may play a role in morphogenesis and thus affect survival.

Analphoid marker chromosomes: Report of two cases. *G.R. Velagaleti¹, S.M. Jalal², T. Hughes³, L.H. Lockhart¹, J.R. Nichols¹, V.S. Tonk⁴.* 1) Dept Pediatrics, Univ Texas Medical Branch, Galveston, TX; 2) Dept. Lab Medicine, Mayo Clinic, Rochester, MN; 3) Brackenridge Children's Hospital, Austin, TX; 4) Dept Pediatrics, Texas Tech Univ, Lubbock, TX.

In spite of recent advances in molecular cytogenetics, marker chromosomes continue to be a diagnostic dilemma. The conventional approaches of special banding techniques and FISH using alpha satellite probes have been the principal methods of their identification. However, at times these methods can be inadequate and may lead to erroneous interpretation. We present two cases in which the unusual marker chromosomes were identified by M-FISH

Case 1 involves a 21-month-old Caucasian male. He presented with dysmorphic facies and a cataract in the left eye. Peripheral blood chromosome analysis showed a mosaic karyotype with 48,XY,+marx2[14]/46,XY[40]. The marker chromosomes were identical in morphology and were nearly the size of a G-group chromosome. Case 2 involved a 5-year-old Caucasian male with hyperactivity and developmental delay. He had minor facial dysmorphism and sub mucous cleft. He also showed a mosaic karyotype 47,XY,+mar[17]/46,XY[7]. The marker chromosome appeared smaller than G-group. The marker chromosomes were C- and AgNOR band negative. FISH analyses with several alpha satellite probes including D13Z1/D21Z1 were negative in both cases. M-FISH analysis, however, in both cases showed that the marker chromosomes are derived from a chromosome 13. Subsequent FISH with wcp13 confirmed these findings.

Since both markers were non-satellited, c-band negative and did not hybridize with alpha satellite probes for chromosomes 13 and 21, it is concluded that these marker chromosomes are analphoid in nature and have a neocentromere. Further studies to localize the neocentromere are in progress. In light of these findings caution is warranted in identification of such marker chromosomes using conventional techniques.

Suspected gonadal mosaicism for isochromosomes 18p and 18q unsubstantiated by FISH analysis of sperm. *M.S. Williams¹, K.D. Josephson¹, N. Gursoy², C. Jackson-Cook²*. 1) Dept. of Pediatrics, Gundersen Lutheran Medical Ctr, La Crosse, WI; 2) Dept. of Human Genetics, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA.

A father had two children (with two different mothers) who had chromosome 18 abnormalities. The first was diagnosed prenatally as having isochromosome (i) of 18q when holoprosencephaly was seen by ultrasound. The second was diagnosed, following an amniocentesis done for advanced maternal age, as having i 18p. A lymphocyte chromosome analysis of the father showed no evidence of i 18p or i 18q mosaicism. However, given the rarity of these two conditions the father was counseled that he might have gonadal mosaicism for i of 18p and 18q which could confer a high recurrence risk for one or the other of these chromosomal abnormalities. To assess this possibility, dual color FISH was performed on sperm collected from a semen sample provided by this man using probes specific for the subtelomeric regions of 18p and 18q. None of the 1000 sperm scored had a FISH pattern consistent with an isochromosome. Furthermore, the sperm aneuploidy frequency for chromosome 18 (0.3 % disomy) was not significantly different from that seen in control males (0.5% + 0.05%). Thus, the sperm population present at the time of semen sampling from this male provided no evidence of mosaicism. This finding suggests that the recurrence risk for this male is low and illustrates the importance of utilizing laboratory confirmation of clinical hypotheses whenever possible.

Duplication of the proximal portion of chromosome 5p. *D.L. Persons, H.H. Ardinger, T.M. Arthur, B.D. Davoren, L.D. Hankins, J.E. Ragona.* University of Kansas Medical Center, Kansas City, KS.

Several reports have described duplications of the entire short arm of chromosome 5 or the distal portion of 5p. Partial trisomy restricted to the proximal region of 5p has been reported in only two cases, one resulting from an insertion and one from a marker chromosome. We report the first de novo direct duplication of the short arm of chromosome 5 involving 5p11-p13.1. The 3 year-old female patient presented with a history of significant developmental delay, macrocephaly, hyperopia, abnormal EEG (without seizures) and feeding problems in infancy. She required oxygen in the first year of life for an undefined lung problem. Height and weight were normal. She had scaphocephaly, sparse slow-growing straight scalp hair, mild scoliosis, joint laxity, eleven pairs of ribs, normal bone age, inverted nipples, tiny umbilical hernia and very soft skin that was thin over the dorsa of the hands. Facial features were reminiscent of Noonan syndrome, CFC syndrome, and Costello syndrome, but the other clinical findings did not support any of these diagnoses. Initial routine chromosome analysis was reported as normal. A follow-up high resolution chromosome analysis (800 band level) revealed a small direct duplication of the proximal portion of 5p with breakpoints at p11 and p13.1. A portion of C-band positive material was also duplicated. Fluorescence in situ hybridization using a whole chromosome paint for chromosome 5 confirmed that the duplication consisted of only chromosome 5 material. Parental chromosomes were normal. Direct duplication of the proximal region of 5p has not, to the best of our knowledge, been previously described. The patient's characteristics were consistent with those that have been reported for whole arm 5p duplications and distal 5p duplications involving the 5p13 band. Generally, duplications involving 5p that are distal to the p13 band have milder phenotypic effects. These findings support the importance of the 5p13 band in causing the severe phenotype observed in many whole arm and partial duplications of 5p.

CGH as a routine procedure in a clinical cytogenetic laboratory : a one-year assessment. *C. Turleau, J.M. Lapierre, G. Joly, M. Prieur, O. Raoul, M.C. de Blois, N. Morichon-Delvallez, P. Gosset, S. Romana, M. Vekemans.* Laboratoire de Cytogenetique, Hopital Necker Enfants Malades, Paris, France.

Comparative Genomic Hybridization (CGH) is able to detect the origin of extra or missing chromosome material in cases of chromosome imbalances. To assess its usefulness as a routine procedure in a clinical cytogenetic laboratory we performed CGH on a total of 55 cases analyzed previously using high resolution R and G banding (HRB). First, we validated the technique on 9 cases with well-defined deletion or duplications. All imbalances were confirmed using CGH. In 16 cases, further characterization of a chromosome rearrangement detected using HRB was necessary: identification of extra or missing chromosome material (5 cases), confirmation of the unbalanced nature of a rearrangement (9 cases) or accurate definition of the breakpoints (2 cases). CGH was useful in all cases. For example, in one case of add(8p), CGH shows an enhancement and diminution of chromosome 8 material confirming a small inv dup(8p); in another case CGH identified an insertion of a 7p segment into the short arm of chromosome 8. In 24 cases, HRB analysis was normal. Among these, 16 cases also had a normal CGH profile. In 8 cases however CGH detected a chromosome imbalance. They comprise 6 patients (4 families) with an unbalanced familial translocation and 2 other unbalanced translocations (1 de novo and 1 of undetermined origin). Finally 6 prenatal cases with a suspected chromosome rearrangement were studied. All had a normal CGH profile. All abnormal results were always confirmed using whole chromosome painting and/or FISH with subtelomeric probes. Interestingly, in our experience all subtle telomeric imbalances were detected using CGH. In conclusion, we believe that CGH could be used as a routine procedure in a clinical cytogenetic laboratory as an alternative to FISH screening using all telomeric probes. Also this technique is able to analyze interstitial chromosome imbalances.

Trisomy 8 mosaicism: cell cycle kinetics and distribution of trisomy 8 and normal cells in embryonic and extra-embryonic tissues. *B.J. Pettit, M. Hummel, L.L. Cook, B.K. Boyd, S.L. Wenger.* West Virginia University, Morgantown, WV.

Amniocentesis performed at 17 weeks gestation due to abnormal maternal serum triple screen identified a fetus with trisomy 8 in one third of metaphase cells while the remaining cells were normal. Trisomy 8 mosaicism is associated with variable clinical manifestations such as mental retardation, deformed skull, prominent forehead, high-arched palate, low-set and/or dysplastic ears, deep palmar and plantar furrows, and reduced joint mobility. The purpose of this study was to determine the level of trisomy 8 mosaicism found within embryonic and extra-embryonic tissues, rates of cell division for the two cell lines, and effect of mosaicism on the phenotype. Tissue samples collected from the baby at birth included cord blood, cord tissue, peripheral blood, and separated placental tissues (chorion, amnion, and villi). The level of trisomy 8 cells in cord blood and peripheral blood was 54%, umbilical cord was 57%, amnion was 43%, and chorion was 8%. Cell cycle kinetics was measured using incorporation of bromodeoxyuridine during 48 hours to determine the number of cell divisions in metaphase cells. Cell cycle kinetics of the cord blood for trisomy 8 cells had 18% 1st division, 25% 2nd divisions, and 57% 3rd divisions; and normal cells had 10% 1st division, 36% 2nd divisions, and 54% 3rd divisions. There was no significant difference in growth rates between the trisomy 8 and normal cells using Chi-squared goodness of fit test. Cell cycle kinetics for other tissues is pending. The infant had bitemporal narrowing, large fleshy ears, hypoplastic nails, deep furrows on palms and soles, first-degree hypospadias, severe cardiac enlargement secondary to pulmonary hypertension, and feeding difficulties. The child expired at 8 weeks due to pulmonary hypertension. Low level of trisomy 8 cells in placental tissue may have allowed the fetus to go to term, while the higher level in patient tissues is probably related to poor outcome.

Is there a mosaic trisomy 12p phenotype? *R. Smith, J.M. Cowan, D.W. Bianchi.* Division of Genetics, Department of Pediatrics, New England Medical Center, Boston, MA.

Constitutional trisomy 12p is rarely observed. It most frequently presents with monosomy for another region as a result of meiotic missegregation of a parental balanced translocation. Delineation of the associated phenotype has been complicated by the concomitant monosomy in most cases; however, de novo cases and those involving monosomy for the short arm of acrocentric chromosomes have permitted the delineation of a "trisomy 12p syndrome" phenotype. Some of the features described include normal to large birth weight, severe motor retardation, hypotonia, prominent forehead with flat facies, hypertelorism, bilateral epicanthus, low nasal root, short upturned nose with anteverted nares, large mouth, prominent protruding lower lip, micrognathia and a short neck. The phenotype of mosaic trisomy 12p has not been clearly defined given that there have been only 3 previously reported cases, all with an overall high level of mosaicism.

We present a fourth case of trisomy 12p mosaicism. Our patient was the 10lb. 4oz. product of a term gestation born to a 37 year old G2P1 mother. Generalized hypotonia with developmental delays was evident by 3 to 4 months of age. At 5 months she was evaluated by genetics and her exam was significant for her large size (all growth parameters greater than the 95th percentile). Her forehead was prominent and bossed. Her palpebral fissures were upslanting. She had a low nasal root with a short upturned nose. Her philtrum was long with a thin upper lip and a protuberant lower lip. Her mouth was wide. She had marked hypotonia. Peripheral blood karyotype analysis revealed 47,XX,+del(12)(q11)[13]/46,XX[7]. At 8 months she was able to sit with support for short periods and did not yet have full head control. An EEG showed no seizure activity.

Comparison of our patient's phenotype with those previously reported suggests that the phenotype of mosaic trisomy 12p is similar to the phenotype of full trisomy 12p. More reports will be necessary to determine whether or not a lower percentage of mosaicism has an effect on this phenotype.

Jumping translocations of 9q onto 14p, 13q and 7q, and pseudo isochromosome 9p results in 9p trisomy

syndrome. *K. Wakui¹, E. Hidaka², M. Ishikawa², M. Ichikawa³, T. Katsuyama², Y. Fukushima¹.* 1) Dept Hygiene & Med Genetics, Shinshu Univ Sch Medicine, Matsumoto, Nagano, Japan; 2) Dept Laboratory Medicine, Shinshu Univ Sch of Med, Matsumoto, Nagano, Japan; 3) Dept Pediatrics, Shinshu Univ Sch of Med, Matsumoto, Nagano, Japan.

We report a 9p-trisomy-syndrome patient with jumping translocations (JTs) of 9q and pseudo iso chromosome 9p. The patient, a 12-year-old girl, was referred to our clinic because of mental retardation and short stature. She was born at 38 weeks' with a birth weight of 2472 g and length of 47 cm. Developmental milestones were slightly delayed; she rolled over at six months, stood alone at 19 months. Her characteristic features were: short stature (132.4 cm, -3.0 SD), microcephaly (47.8 cm, -3.0 SD), long face, high nasal bridge, long nose, short philtrum, prominent ears, short 5th fingers, brachymesophalangea, and kyphosis. Cytogenetic analysis on both lymphocytes and fibroblasts showed complicated mosaic rearrangements. All cell lines had pseudo isochromosome 9p confirmed by FISH analyses using wcp 9 and 9p telomeric probes. Added to this, JTs of 9q onto 14p (60% of cells), 13q (25%), 7q and other chromosomes were observed. The parents had a normal karyotype. The patient did not suffer from hematologic malignancy. JTs is defined as mosaic chromosomal rearrangements characterized by the relocalization of the same donor chromosome segment to several recipient chromosomes. JTs is commonly observed as an acquired form of chromosomal rearrangement in neoplastic cells from cancer patients, but constitutional forms of JTs are very rare. To our knowledge, this is the first case of JTs of 9q results in 9p trisomy syndrome. The breakpoints of the recipient chromosomes in JTs including our case occur in areas rich in repetitive DNA (telomeric, centromeric, and nucleous organizing regions). It may point to resolving the mechanism of constitutional forms of JTs.

Abnormal sex development and autism in a 46,XX female with 9p24.1-pter chromosomal rearrangement. L.

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We describe a female patient harbouring an unbalanced de novo 46,XX,t(9;18) chromosome complement and multiple congenital abnormalities. The child presented with profound mental retardation, autistic features, dysmorphic face and severe developmental delay (-4SD). Puberty delay was also present: stage 2 at 15.5 years of age and absence of the first periods. Ultrasounds investigation showed the presence of an infantile uterus, and two small apparently normal ovaries with immature primordial follicles. Further endocrinological investigations showed the presence of an auto-immune Hashimoto thyroiditis with raised plasma levels of antimicrosomal antibodies. The patient presented with behavioural anomalies including autistic features, automutilations and stereotypic gesticulation. Karyotype analysis showed a homogenous de novo 46,XX,9p+ chromosome complement. The extra material present at the tip of the short arm of the chromosome 9 could be identified as a chromosome 18 fragment after MULTIFISH analysis. The chromosomal abnormality resulted the loss of the terminal 9p region (p24.1-pter). Deletions of this region in 46,XY patients have been intensively investigated, and a gene responsible for sex determination has been mapped at this level. However, little is known about this type of chromosomal abnormality in 46,XX individuals. Two other female 46,XX patients with chromosomal rearrangement involving 9p24.1 were described, both presenting with abnormal gender identity, gender dysphoria and autistic features (unpublished data). It is conceivable that a common gene involved in sex determination might have been disrupted by the chromosomal rearrangements seen in these patients and the different male and female abnormal phenotypes might be determined by a variable expression in XY and XX individuals of this putative gene. The presence at the same locus of a gene responsible for autism is also discussed.

Three siblings have three different imbalances following 3:1 malsegregation of a maternal translocation. *R.C. Weaver, C. Jackson-Cook, J. Bodurtha.* Human Genetics, Virginia Commonwealth Univ., Richmond, VA.

The majority of reciprocal translocations malsegregate to produce only one type of viable imbalance in humans. We report a very rare family in whom three different chromosomal imbalances were noted in three siblings. The mother (G4A1P3) of this family carried a balanced translocation between chromosomes 12 and 21 [t(12;21)(p13;q21)]. She was ascertained following the birth of her first child (currently 15 yo), who had Down syndrome due to an interchange trisomy following 3:1 malsegregation of the maternal translocation. Her second (currently 8 yo) and third (currently 6 yo) children (both of whom were diagnosed prenatally) had imbalances resulting from the complementary 3:1 tertiary interchange products leading to partial monosomy [der(12)t(12;21)] and trisomy [47,XX,+der(21)t(12;21)], respectively. Thus, the imbalances in her children include trisomy 21 (child 1); partial monosomy for distal 12p and proximal 21q (child 2); and partial trisomy for proximal 21q and distal 12p (child 3). As expected from the different imbalances, the phenotypes of the children vary. The oldest sibling has findings typical of those noted in children with Down syndrome. The second child (partial monosomies) has developmental delay, severe growth retardation, micrognathia, mild hearing loss, broad tipped nose, broad pectus, heart murmur, and an undescended testicle (which has been repaired surgically). The youngest sibling is mildly affected, having a prominent widow's peak, a narrow vermilion border of the upper lip, and no apparent developmental delay. This translocation is unique in that multiple imbalances resulting from the predicted 3:1 malsegregation are compatible with life. Moreover, this family has presented a unique genetic counseling dilemma in identifying an appropriate support group(s) and calculating recurrence risk.

Clinical Manifestations associated with Del(2)(q32.1q33.1). *K.A. Smyth, R. Babul-Hirji, D. Chitayat.* Division of Clinical Genetics, The Hospital for Sick Children, Toronto, ON, Canada.

Over 20 cases of interstitial deletion chromosome 2q have been reported, the most common being del(2)(q31q33). Common manifestations among these children include low birth weight, developmental delay/mental retardation, microcephaly, cleft palate and dysmorphic facial features. Renal hypoplasia, agenesis of the corpus callosum, acetabular hypoplasia, absent ribs, seizures, equinovarus deformities of the feet and iris coloboma have also been reported.

We report on a 14 year old girl with a de novo del(2)(q32.1-33.1) and global developmental delay. The patient was born at 32 weeks with a birth weight of 2kg(50%ile). The pregnancy and neonatal course were uncomplicated. She had valgus deformity of both feet, corrected with bracing. At 14, her height was 135cm(<3%ile), weight 37kg(<3%ile), and head circumference 55.5cm(50-98%ile). Dysmorphic features included brachycephaly, high forehead, frontal bossing, narrow bitemporal diameter, prominent supraorbital ridges, deep set eyes, hypoplastic malar areas, prominent maxilla with malocclusion and wide spaced teeth, and narrow vermilion borders. She had lumbosacral scoliosis of 17-20°, 12 ribs and 4 non-rib bearing lumbar type vertebral bodies, and spina bifida occulta at L5. Her visual acuity was 20/20 with significant myopia and astigmatism bilaterally. Her upper limbs showed dislocation of the radial head, hyperextensibility, and arachnodactyly with wrinkling over the palms. Her lower limbs revealed a "saddle" gap and hyperextensible knees. A brain CT scan revealed mild to moderate ventriculomegaly and the anterior horns of the ventricles were split apart. Comparison of our patient with the literature confirms the association of this deletion with developmental delay and characteristic facies. This report lends support to the association of this deletion with skeletal abnormalities, and is the first report of ventriculomegaly associated with this condition. The genes likely to be important within this region include the distal-less homeobox genes DLX1 and DLX2 (2q32), and the gamma crystallin genes (2q32-35). Further investigation is being done to determine if these genes are deleted.

Genetics of Klippel-Trénaunay Syndrome (KTS), a vascular disease. A. Sadeghpour¹, M. McCane², G. Hoeltge², D. Driscoll³, Q. Wang¹. 1) Center for Molecular Genetics, Cleveland Clinic Foundation, Cleveland, OH; 2) Department of Clinical Pathology, Cleveland Clinic Foundation, Cleveland, OH; 3) Section of Pediatric Cardiology, Mayo Clinic, Rochester, MN.

Klippel-Trénaunay Syndrome (KTS) is a congenital vascular disease which is defined as a combination of capillary, venous and lymphatic malformation associated with hypertrophy of affected tissues. The majority of KTS cases are sporadic. However, a few familial cases have been reported and also several studies have shown that frequency of hemangiomas or varicose veins are higher in the first degree relatives. Moreover, two cytogenetic changes in KTS patients: t(5;11) (q13.3;p15.1) and t(8;14) (q22.3;q13) have been identified. Therefore, these findings suggest that genetic factors contribute to pathogenesis of KTS. Our research goal is to identify more cytogenetic abnormalities in KTS patients since this strategy is very powerful in finding the disease-causing gene(s). Identifying and characterizing the KTS gene(s) will help us to understand the molecular mechanisms underlying the vascular morphogenesis. Blood samples have been received from KTS patients and their family members. Family studies have shown that more than 50% of KTS patients have other family members affected with various vascular disorders. Karyotyping (50 patients) and fluorescence *in situ* hybridization (FISH) have been used to identify and localize cytogenetic changes. Several cytogenetic abnormalities with chromosomal mosaicisms have been found: Mos 46, XX/47, XXX; Mos 46, XX/45, X; and 46, XY/47, XY,+r. All the findings point out to a hereditary pattern and possibility of genetic factor(s) for KTS.

Characterization of a familial acentric marker chromosome identified at amniocentesis. *J. Winters*¹, *M. Lipson*², *M. Dolliver*², *S. Owen*¹, *S. Schwartz*³. 1) Genetics Dept, Kaiser Permanente Northern California, San Jose, CA; 2) Sacramento, CA; 3) Ctr for Human Genetics and Dept of Genetics, Case Western Reserve Univ and Univ Hospitals of Cleveland, OH.

Acentric marker chromosomes (generally defined as lacking identifiable a-satellite sequences) have been reported in a number of cases. These markers have been found to originate from several chromosomes and are almost always de novo in origin. We have identified and studied a familial case of a prenatally ascertained acentric marker chromosome. A 38-year-old, G3P1SAB1 woman had an amniocentesis for advanced maternal age. The fetal karyotype was found to be 47,XY,+mar in all 15 colonies examined. Fluorescence in situ hybridization (FISH) studies were performed, and the marker chromosome was found to be negative for the following probes: DXZ1, DYZ3, D15Z1, SNRPN, PML, and a pancentromeric probe (thereby characterizing this marker as an acentric chromosome). Studies of parental blood were performed, and the mother was found to have an apparently identical non-mosaic marker chromosome (47,XX,+mar). She is phenotypically normal (with a negative family history for mental retardation and birth defects), indicating that there are non-centromeric portions of the genome which are not detrimental when present in three copies. Besides probing for a-satellite sequences, CENP (centromere-associated proteins) antibody studies are also used to characterize neocentromeres (non-a-satellite sequences which have centromeric activity). Neocentromeres generally bind CENP-B (which is present at inactive and active centromeres) as well as CENP-C and -E (which are present only at active centromeres). Studies to determine the chromosomal origin and CENP status of this marker are currently underway, as are family studies to identify other carriers. This case demonstrates that (1) detectable a-satellite sequences are not necessary for faithful mitotic or meiotic replication and segregation and (2) partial aneuploidy for some non-centromeric regions of the genome is not necessarily associated with phenotypic abnormalities. To our knowledge, this is the first reported case of a supernumerary familial acentric marker chromosome.

An insertional translocation in an infant with two common fragile sites in the mother. *U. Tantravahi¹, D. Abuelo².*

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Autosomal fragile sites have been implicated in possible predisposition to chromosome breakage and rearrangements in meiosis. We describe a patient with an insertional translocation involving chromosomes 3 and 16 whose mother had fragile sites at both of the break points. Case Report: Amniocentesis done in another country because the fetus had hydrops, pleural effusions and polyhydramnios identified a deletion in chromosome 3p13-p14. The infant was born in Providence, RI at 36 weeks and had dysmorphic features consisting of asymmetric, downslanting palpebral fissures, wide nasal bridge and unusual pinnae. She also had moderate hypotonia and an atrial septal defect was detected. At 8 months of age, psychomotor development was delayed. G-banded karyotype analysis of the patient revealed an insertional translocation with break points in chromosome 3 at p14 and p21. This segment from chromosome 3 was inserted at the q22 region of chromosome 16. These results were confirmed by FISH analysis using whole chromosome paint probes for 3 and 16. The break points at 3p14 and 16q22 are associated with common fragile sites. Although both parental chromosomes were normal, the maternal karyotype revealed a fragile site at chromosome 3p14 without induction in 32% of the cells. Induction with Aphidicolin and Distamycin showed increased expression of fragile sites at 3p14 and 16q22 in the mother compared to the normal background levels. These results suggest that the fragile sites on these two chromosomes in the mother may have contributed to the chromosome rearrangement seen in the child. The clinical symptoms may be due to loss of genetic material that may have occurred in the process of chromosome rearrangement, position effect of genes involved in the rearrangement or breaks in the critical genes.

Miscarriages: Cytogenetic studies and recurrence risks. *P.N. Rao*¹, *P.M. Tsuribe*², *E.O. Rodini*³, *L.V. Rodrigues*⁴. 1) Pathology/Lab Med, Univ California, Los Angeles, Los Angeles, CA; 2) ALAG/UNESP, Bauru, SP, Brazil; 3) ALAG/UNESP, Bauru, SP, Brazil; 4) CEPS/USC, Bauru, SP, Brazil.

The global rate of spontaneous abortions is between 33 and 67%. The chromosomal abnormalities constitute the commonest causes. About 50% of fetal deaths, in first trimester of clinically recognized pregnancy, are chromosomal abnormalities. Spontaneous abortions behave like a process of "natural selection", eliminating abnormal conceptuses. In the absence of this mechanism, the percentage of newborn with malformations would rise of 3 to 12%. We studied a sample of 752 Brazilian women during reproductive age (18 to 40 years) to verify the incidence, recurrence risks and etiology of spontaneous fetal loss. 148 of them presented with at least one miscarriage. The incidence of miscarriage was 19,68%. Recurrence risks were estimated as following: 23.6% for a second miscarriage (0.1680-0.3050), and 40% for the third miscarriage (0.2377-0.5623). We performed cytogenetic studies (C,G, and Nor banding) on the products of conceptions (POC), using two methods. The first involved culture of stroma of CVS and the second cytotrophoblast cells to obtain direct chromosomal preparations. The results showed: 47,XY,+13(8.7%); 47,XY,+20; 47,XX,+22, 47,XX,+18; 47,XY,+10; 47,XX,+6; 45,XY,-22; 45,XX,-13; 46,XX/47,XX,+9 (4.3% each one), and 69,XXX(13%). Parental karyotypes showed polymorphism in 3 cases (16qh+), and inversion inv(9)(p11q12) in 2 cases. Miscarriages occurred most frequently in the 8th week of gestation. Average maternal age at miscarriage was 28.5 years. About 27.5% were above 35 years old. In women younger than 35 years old, chromosomal anomalies were observed in 51.7%, while in women 35 years or older 63.6% of the POC's had chromosomal abnormalities. These results show the relevance of the genetic counseling and prenatal diagnoses in mothers 35 years or older, and show the increase in the recurrence risks after the first miscarriage.

Biparental Familial Inheritance of Two Unrelated Structural Abnormalities Involving Chromosome 11 and 22 in a Two Generation DiGeorge Syndrome Family. *M.J. Sutcliffe^{1,2}, O.T. Mueller^{1,2}, J. Ranells², D. Dumont¹, D. Conforto¹, J.A. McFarland¹, F. Mawani¹, G. Jervis², A. Mahmoud².* 1) Clinical Genetics Laboratories, All Children's Hospital, St Petersburg, FL; 2) Department of Pediatrics, University of South Florida, Tampa, FL.

A six month old Caucasian female with a history of Tetralogy of Fallot diagnosed at 2 months, hypotonia, delayed motor development and mild facial asymmetry was found to have a microdeletion in the DiGeorge/Velo-Cardio-Facial Syndrome (DIG/VCFS) region of chromosome 22q11.2 by Fluorescence In-Situ Hybridization (FISH) analysis. GTG banding revealed, in addition, a paracentric inversion of chromosome 11. Since translocation (11;22) is well documented in familial DIG/VCFS, parental chromosome analysis was recommended. Pedigree analysis revealed a paternal history of coarctation of the aorta and learning disability. FISH analysis confirmed the DIG/VCFS microdeletion in the father, however, no rearrangement of chromosome 11 was noted. DNA microsatellite analysis identified that the deletion was denovo in the father of the proband and originated on the grandpaternal chromosome 22. Inversion (11)(q13.3q25), the same as observed in the infant, was identified in the maternal karyotype. Paternal grandparents have normal karyotypes and an investigation of maternal grandparents is currently underway. DIG/VCFS microdeletion has been described in combination with Turner, Down and Fragile X Syndromes. To our knowledge this is the first case of familial DIG/VCFS with a second independent structural abnormality. Familial DIG/VCFS has been shown to have significant inter and intra familial clinical variability consistent with the hypothesis of contiguous gene syndromes. In view of the apparent increased clinical severity between father and daughter in this family, delineation of the size of the deletion at the molecular DNA level will be presented and may indicate a genetic basis for this observation and provide important patient management information.

Genomic sequencing and transcript identification over a 700kb region in human 6p24 containing three translocation breakpoints associated with orofacial clefting. *J. Ragoussis¹, S. Knaggs¹, G. Mirza¹, I. Macris¹, R. Stephens¹, A. Mungall².* 1) Div Medical & Molecular Gen, Kings College, Guy's Hosp, London, England; 2) The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, England.

We have previously presented three patients exhibiting the phenotype of orofacial clefting (OFC) associated with balanced translocations involving the 6p24 region (Davies et al 1995 & 1998), suggesting that a gene present in this region, may if disrupted result in OFC. In order to identify such a gene, we first completely cloned the region containing the three breakpoints in YAC, PAC, BAC and cosmid clones, by using YAC fragmentation and genomic clone contigging techniques. The three breakpoints have been located within a 500kb long segment by a combination of fluorescent in situ hybridisation techniques including fibre FISH. At present, sequencing of a 700kb region including the 500kb breakpoint cluster is close to completion. The systematic use of a comprehensive set of sequence analysis tools and RACE techniques lead to the isolation of a single novel transcript expressed in the human fetus which maps to a critical region between two of the breakpoints. Full characterisation of the gene is in progress and this will hopefully lead to an understanding of the molecular basis of the associated developmental defects. Furthermore it will possibly lead to an improved understanding of molecular events during craniofacial development and serve as a candidate gene for cleft lip and palate. Davies AF, Stephens RJ, Olavessen Mg, Heather L, Dixon MJ, Magee A, Flinter F, Ragoussis J Evidence of a locus for orofacial clefting on human chromosome 6p24 and STS content map of the region. *Hum Mol Genet* 1995;4:121-84 *Hum Mol Genet* Davies AF, Imaizumi K, Mirza G, Stephens RS, Kuroki Y, Matsuno M, Ragoussis J. Further evidence for the involvement of human chromosome 6p24 in the aetiology of orofacial clefting. *J. Med. Genet* 1998;35:0-4 *J. Med. Genet* 35.

Molecular cytogenetic characterization of partial duplication 21q. *G.D. Wenger¹, E. Hamelberg¹, T. Depinet¹, J. Labanowska¹, J.R. Korenberg², J.M. Gastier¹.* 1) Dept. of Laboratory Medicine, Children's Hospital, Columbus, OH; 2) Molecular Genetics Laboratory, Cedars-Sinai Medical Center, Los Angeles, CA.

We report a patient with some features of Down Syndrome (DS) and partial duplication of chromosome 21q. GTG-banded analysis of peripheral blood chromosomes revealed a subtle abnormality: one chromosome #21 with additional material added to the stalk/satellite region. At the time of initial analysis, no information regarding indication for study accompanied the sample. To discriminate between a polymorphic variant and significant cytogenetic abnormality, NOR-staining and FISH using the b acrocentric probe and WCP21 were performed. NOR staining was positive; the additional material did not hybridize with the b acrocentric probe but did hybridize with WCP 21, indicating duplication of chromosome 21 sequences. When clinical information was obtained, the patient, a 22-year-old female, was reported to have facial and physical appearance suggestive of DS, no known cardiac anomaly, and normal intelligence. FISH with commercially available probes indicated the duplicated region to be distal to D21S65 and to include the loci D21S55, D21S259/S341/S342, and unique subtelomeric sequences (D21S1219/S1220). Additional FISH studies using the P1 probes 82F5, 140K16, and 850B12 failed to indicate the inclusion of any of these sequences in the duplicated region. The duplicated region therefore apparently extends from just centromeric of D21S55 through the telomere. The physical map of chromosome 21 allows molecular definition of loci included in such rare cases of partial trisomy; study of such patients contributes to our understanding of the loci responsible for the distinct features of DS and their pathophysiology.

Cytogenetic and molecular characterization of unbalanced chromosomal rearrangements in two patients with Wolf-Hirschhorn syndrome. *C. Sreekantaiah, D. Kronn.* New York Medical College, Valhalla, NY.

Wolf-Hirschhorn syndrome (WHS), a multiple malformation syndrome characterised by developmental and mental defects, results from a hemizygous deletion of the distal short arm of chromosome 4 (4p16.3). The deletion occurs either as an isolated deletion or as a result of segregation of a reciprocal translocation. The deletions can range from one-half of the short arm to cytogenetically undetectable. WHS is considered to be a contiguous gene syndrome with an undefined number of genes contributing to the phenotype because of the complex and variable expression of the disorder. We present the cytogenetic and molecular cytogenetic findings in two cases of Wolf-Hirschhorn syndrome. Cytogenetic and fluorescence in-situ hybridization (FISH) analyses of the patients were performed prenatally on amniotic fluid specimens obtained from amniocentesis performed in the third trimester for intrauterine growth retardation and on peripheral blood obtained postnatally. Karyotypic analyses of the parents' were carried out on peripheral blood cultures. In both patients cytogenetic analyses revealed additional material of unidentifiable origin on 4p. High resolution banding analyses and FISH analyses helped to characterize the rearrangements. Analysis of patient KB and her parents revealed that the patient had inherited an unbalanced segregation product [der(4)t(4;5)(p15.2;q33.1)] from a constitutional balanced translocation in her mother [t(4;5)(p15.2;q33.1)]. The rearrangement in patient AB involved a de novo deletion and inverted duplication of 4p; der(4)del(4)(p16.2)dup(4)(p15.33p14). His parents were karyotypically normal. Shared clinical features in both patients included growth retardation, both were small for gestational age, microcephaly, multiple dysmorphic features and significant developmental delay. Certain unique clinical features were also present. Although different mechanisms can contribute to the origin of WHS, the clinical manifestations of WHS depend on the extent of the deleted segment.

Cryptic translocations with acrocentric short arms identified by FISH. *J.H. Tepperberg¹, I.K. Gadi¹, P.N. Mowrey¹, K. Phillips¹, J. Barnum¹, C. Costanzo¹, T. Creeden², M. Hummel³, P.R. Papenhausen¹.* 1) Cytogenetics, LabCorp, RTP, NC; 2) Div Clin Genet, Saint Peter's Univ Hosp, New Brunswick, NJ; 3) Ped/Genet, West Virginia Univ, Morgantown, WV.

Translocations of small, distal chromosome regions to the p-arms of acrocentric chromosomes are often difficult to detect by routine cytogenetics. Failure to identify the rearrangement can lead to a misdiagnosis. We report two cases, showing cryptic rearrangements with the short arm of acrocentric chromosomes. The first case involved a healthy 35yo female, G4P1, referred for amniocentesis because of AMA. Family history was negative. The fetal karyotype analysis showed an apparent deletion of distal chromosome 17p13. Fetal U/S and parental chromosome analyses were normal. Preliminary discussions with the parents, prior to the completion of the FISH study, centered around Miller-Dieker syndrome. A LIS1 DNA probe specific for chromosome 17p13 surprisingly showed probe hybridization with the distal chromosome 21p suggesting an apparently balanced rearrangement. NOR staining of the derivative chromosome 17 was positive. The second case involved a newborn full term female, born to a 23yo G1P1 with MR. Birth wt was 9 lbs 15oz, length 19 in., and HC 37cm. Apgars were 4 at 1 min and 8 at 5 min. Anomalies included horseshoe kidneys, Dandy Walker malformation, ASD and PDA. The infant was mildly dysmorphic with upturned nose, tented mouth, long narrow feet and decreased tone. Chromosome studies showed a G-dark crescent band on the stalk region of chromosome 15p. FISH and karyotype analyses suggested distal 3p origin. This derivative chromosome 15 was correctly identified albeit it could have possibly been interpreted as a normal variant 15p. Furthermore, this case represented pure partial trisomy 3p syndrome. The first case demonstrates the absolute necessity of follow-up by molecular cytogenetic analysis in prenatal cases with apparent terminal deletions. The second case illustrated the importance of investigating the origin of satellite associated G-dark bands, in those cases referred with an abnormal phenotype and/or positive family history.

Jacobsen syndrome : four Korean children with thrombocytopenia or neutropenia. *E.J. Seo, S.M. Ahn, J.Y. Lee, H.W. Yoo.* Medical Genetics Clinic & Lab, Asan Medical Center, Seoul, Korea.

Jacobsen syndrome is caused by terminal deletion of the long arm of chromosome 11. Patients with this syndrome have breakpoints in 11q23.3-11q24.2 and typical features including psychomotor and growth retardation, trigonocephaly, dysmorphic faces, ocular anomalies, cardiac defects, digit anomalies, and thrombocytopenia or pancytopenia. We have evaluated four Korean children with terminal deletion of 11q. Two patients had a breakpoint in 11q23.3 which may be related to the FRA11B fragile site. Remaining two patients showed a terminal deletion to distal 11q24. These patients had cardiac defects and dysmorphic facial features such as trigonocephaly, high-arched palates, and low-set ears. Strabismus, foot deformities, imperforated anus, and cryptorchidism were also found. Severe to moderate thrombocytopenia was observed in three patients. Neutropenia was detected in two patients. All patients revealed mild anemia. There are two candidate genes known to map to 11q24-25 that are likely to play some role in hematopoiesis: Ets-1 and nuclear-factor-related-kB (NFRKB) gene. Deletion of these genes appears to cause insufficiency for normal hematopoietic differentiation or development and result in thrombocytopenia or pancytopenia.

De novo chromosomal abnormalities in prenatal diagnosis. *S.Y. Park¹, Y.M. Kim¹, J.M. Kim¹, J.W. Kim¹, B.Y. Lee¹, M.Y. Kim², J.Y. Han², H.M. Ryu².* 1) Lab Medical Genetics, Samsung Cheil Hosp, Seoul, Republic of Korea; 2) Department of Obstetrics and Gynecology, Samsung Cheil Hospital & Women's Healthcare Center, School of Medicine, Sungkyunkwan University, Seoul, Korea.

We report 17 cases of de novo chromosomal abnormalities detected in 4709 cases of prenatal cytogenetic analysis which had been performed in Samsung Cheil Hospital from 1995 and 1999. Of the 17 cases, 5 were balanced reciprocal translocations and 12 were unbalanced translocations characterized as deletion, addition or marker. The indications for karyotyping of present cases were based on ultrasound abnormal findings (8), high risk of Down syndrome at prenatal screening test (4), and advanced maternal age (5). As a result, we observed unbalanced translocation such as partial chromosomal deletion or addition in 7 cases of ultrasound abnormal findings. Also, one case of translocated Down syndrome and two cases of mosaicism with marker detected in suspected cases for Down syndrome on prenatal screening test. Additionally, in cases indicated by advanced maternal age, 3 were balanced translocations and 2 were unbalanced translocation of chromosome 18 located in short arm and long arm, respectively. Although de novo chromosomal translocation is balanced, it could result in phenotypic abnormalities due to cryptic deletion and duplication or malfunction of gene near the region of breakpoint. We found 2 cases of balanced translocation in ultrasound abnormalities. Taken all together, it became important that the precise diagnosis and adequate genetic counselling is necessary for de novo chromosome abnormalities in cytogenetic analyses prenatally. Thus, we aim to elucidate more accurate characteristic of de novo chromosomal abnormalities using FISH, CGH, and high-resolution banding / reverse banding.

Microscopic and submicroscopic deletion 22q13.3 found in patients referred for Angelman syndrome. *P. Van Tuinen¹, M. Lubinsky^{1,2}, C. Brooks¹, M. Emery¹, J. Grignon¹, P. Miller¹, P. Schalk¹, H. Radtke².* 1) Pathology, Clinical Cytogen Lab, Medical Col Wisconsin, Milwaukee, WI; 2) Children's Hospital of Wisconsin, Milwaukee, WI.

Features observed in Angelman syndrome (AS) (lack of expressive speech, developmental delay) are commonly found in patients with deletion 22q13. There appears to be significant overlap in the major features of these syndromes, with the apparent result being frequent requests for diagnostic studies for the more widely known AS. Precht et al. (J. Med. Genet. 35: 939; 1998) serendipitously found a 22q13 deletion in a patient whose AS studies were negative. Using FISH, this deletion was evident by the absence of a signal for distal 22q13 normally generated by a control probe for velocardiofacial/diGeorge syndrome (VCF/DGS). The authors suggested that 22q13 deletion be considered in patients exhibiting these features, and that a subset of patients suspected of having AS may have deletion 22q13. Following our finding of a visible deletion 22q13 in a patient referred for AS, we began performing simultaneous FISH using an artificial mixture of AS probe (D15S10 and control probe PML) and VCF/DGS probe (TUPLE1 and control probe ARSA). In the last 9 months of operation, 13 patients have been referred to our lab for AS testing. Of these, two had deletion 15q12 with D15S10; the rest were negative by further methylation testing. One patient had a visible deletion 22q13.31, while another with normal high resolution cytogenetic as well as D15S10 studies was deleted for ARSA. A third patient referred only for suspicion of "chromosome abnormality" had add(22)(q13.3). While parental specimens were not forthcoming, loss of material from 22q13 was presumed based on translocation of unknown foreign material to this region. The small sample size notwithstanding, our experience in detecting microscopic and submicroscopic deletion 22q13.3 in patients referred for Angelman syndrome affirms the study of Precht et al., and argues for a multiplex FISH approach in working up patients referred for AS.

Sperm aneuploidy and semen quality in a non-clinical cohort of healthy non-smoking men. *E. Slotter*^{1,2}, *B. Eskenazi*³, *X. Lowe*¹, *K. Kim*¹, *F. Hill*¹, *S. Kidd*³, *J. Nath*², *D. Moore, II*⁴, *A.J. Wyrobek*¹. 1) Bio Biotech Res Prog, Lawrence Livermore National Laboratory, Livermore, CA; 2) Genet Dev Biol Prog, West Virginia Univ, Morgantown, WV; 3) School Public Health Univ California, Berkeley, CA; 4) Pacific Medical Center, San Francisco, CA.

Although previous studies have shown associations between sperm aneuploidy and semen quality among infertile men, there is little information for men with no reproductive health problems. The objective of this research was to investigate the relationship between semen quality and sperm aneuploidy in a large non-clinical cohort of healthy men: 90 non-smokers, aged 20-80y, with no history of infertility or reproductive problems. Semen quality was evaluated using double-blinded analyses of semen volume, sperm concentration, % motility, % progressive sperm averaging 2.9mL (range: 0.3-7.5), 147×10^6 sperm/mL (range: 13-575), 40% (range: 6-83), 22% (range: 0-68), respectively. The frequencies of aneuploid sperm were determined using multicolor FISH with probes for chromosomes X, Y and 21 (a total of 961,097 sperm were evaluated). The mean frequency of total disomic sperm (i.e., sum of X-Y, Y-Y, X-X, and 21-21) was 31.0 per 10^4 (range: 7-106), and the frequency of diploid sperm was 13.1 per 10^4 (range: 1-85). Frequencies of total disomic and diploid sperm were correlated among specimens ($r=0.44$, $p<0.0001$). However, there was little overall evidence for any associations between sperm aneuploidy and the parameters of semen quality measured in this study. Specifically, there were no significant correlations between semen volume or sperm concentrations versus total disomic or diploid sperm. However, we observed a positive association between disomy 21 and sperm motility ($r=0.29$, $p<0.005$). Thus, this study, the largest to date, has found no evidence for an association between poor semen quality and increased frequencies of genetic damage in sperm in a non-clinical population. [Work performed under the auspices of the US DOE by LLNL, contract W-7405-ENG-48, and funding from NIEHS Superfund P4ZES04705 and WVU].

Spectral karyotyping (SKY™) for identification of supernumerary marker chromosomes in constitutional prenatal and postnatal studies. *B.J. White, V. Sulcova, A. Anguiano, K. Hoang, S. Wang, K.S. Reddy.* Dept Cytogenetics, Quest Diagnostics, Inc, S J Capistrano, CA.

In many cases with supernumerary marker chromosomes, it is impractical to apply FISH probes specific for each individual chromosome to determine marker origin. The application of multicolor spectral karyotyping (SKY) permits identification of markers found in prenatal and postnatal studies. SKY paint probes (Applied Spectral Imaging, Carlsbad CA) were used in conjunction with the SKY Vision Cytogenetic Workstation for analysis of a minimum of 5 metaphases from 20 such cases. All markers identified by SKY were confirmed by traditional FISH methods such as chromosome painting and centromeric probing. Of the 20 completed studies, 9 were prenatal and 11 postnatal. The postnatal cases ranged in age from newborn to 58 years. Markers originating from acrocentric chromosomes are underrepresented in this series because satellited markers were generally identified by conventional FISH studies. Identification of those included in this study was accomplished in 16 of 20 cases (80%), and in 4 cases origin could not be determined. Marker derivation included chromosomes 4, 9, 15, 17, 19 (1 case each), 1, 2, 8, 22 (2 cases each) and 18 (3 cases). In a previous communication, we suggested that the practical resolution limit of SKY is approximately one band visible at 500 bands per haploid karyotype. It is likely that the small markers which could not be characterized by SKY had one band or less, or no euchromatin material. It is possible that centromeric material adjacent to a minimum amount of euchromatin may yield equivocal signals precluding positive identification. Identification of markers by SKY will build useful information for clinical correlations. Further documentation of findings associated with the various markers will improve genetic counseling in these clinical situations.

Identification of an unusual i(Y)q chromosome with no detectable DYZ3 centromeric sequence. *J.G. Assumpcao¹, N.L.V. Campos², M.I. Melaragno³, A.T. Maciel-Guerra², M.P. De Mello¹.* 1) CBMEG, Univ. Estadual de Campinas, Campinas, São Paulo, Brasil; 2) Dept. Genética Médica, FCM, Univ. Estadual de Campinas, Campinas, São Paulo, Brasil; 3) Disciplina de Genética, Dept. de Morfologia, UNIFESP/EPM, São Paulo, Brasil.

An 18 year old female was ascertained because of primary amenorrhea and hypogonadism. Chromosome analysis from peripheral blood lymphocytes revealed a non-mosaic 46,X,+mar constitution. G and Q banding patterns indicated that the marker could be an i(Yq), however there was no detectable primary constriction. C banding revealed bright bands on both extremities, which could be due to the presence of the distal heterochromatic portion normally present in Yq. Molecular studies proved the Y-origin of the marker. Deletion mapping analysis with Y-specific STS showed that the marker lacked Yp and centromeric (DYZ3) sequences, whereas all STS tested for Yq presented positive PCR amplification. FISH experiments with X and Y centromeric probes showed no hybridization signals. The marker i(Yq) chromosome is found in 100% of the cells, therefore it is mitotically stable despite the absence of both DYZ3 centromeric sequence and a visible primary constriction. The type of chromosome we report here seems to have adopted a new centromere and it raises the question of what DNA sequences act as a functional centromere in the absence of alpha-satellite DNA.

Response to growth hormone therapy in a patient with a 15q deletion and normal levels of endogenous growth hormone: a challenge to conventional wisdom. *T. Marini, J. Sullivan, R. Naeem, E. Reiter.* Departments of Pathology and Pediatrics, Baystate Medical Ctr, Springfield, MA.

We report on an 11 year old girl originally referred for short stature at age 3 years. She presented as an extremely small child (-5.0 SDS) in whom no obvious endocrinologic diagnosis was made. Previous cytogenetic studies identified a de novo 15q deletion, 46,XX,?15qter. Parental karyotypes were reportedly normal. Subsequent cytogenetics and fluorescent in situ hybridization (FISH) studies confirmed the previously identified deletion at locus D15S396 on one chromosome 15q. Further FISH studies using a +15 coatasome (total chromosome paint) revealed no evidence of any cryptic translocation. A revised karyotype of 46,XX,del (15) (q26.2) .ish del (15) (q26.2) (D15S396-),wcp15+ was reported. Previous cases of ring chromosome 15 and 15q terminal deletions, presenting with growth failure, have suggested that the growth failure is related to loss of one insulin-like growth factor I receptor (IGF1R) gene located at band 15q26. The mechanism for this relationship, however, is unclear, as these patients reportedly produce adequate amounts of growth hormone and presumably still have one intact and functioning IGF1R gene. Previously, growth hormone therapy has not been a consideration for these patients given their normal levels of endogenous growth hormone. Despite normal endogenous levels of growth hormone, our patient was treated with growth hormone therapy for the past 7.5 years because of her extreme short stature. Against the expectations of conventional wisdom, she has shown a good response to exogenous growth hormone rising in height to about -2.5 SDS. The reason for this response is puzzling and may be the result of some epigenetic effect on the IGF1R gene or perhaps some synergistic effects involving other non-IGF genes related to growth. Regardless, our results suggest that similar cases of 15q anomalies, as well as patients with idiopathic growth failure, may benefit from treatment regimens previously reserved only for patients demonstrating low levels of endogenous growth hormone.

Centromeric heterochromatin packaging and centromeric protein binding. *Y.C. Li¹, C. Lee², S.Y. Li¹, C.C. Lin^{1,3}.*

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The ubiquitous presence and specific chromosomal localization of satellite DNA in diverse mammalian genomes imply that satellite DNA may play a role in centromere structure and function. Although in rare occasions, certain mitotically stable marker chromosomes which appeared to be lacking of satellite DNA still retain centromeric function through some type of epigenetic effect. It is postulated that such an epigenetic effect has indeed achieved some degree of proper chromatin packaging and higher order structure which are needed for kinetochore reassembling during mitosis.

The water deer (*Hydropotes inermis*) has two types of centromeric heterochromatin which can be resolved by FISH analysis using cervid satellite I and satellite II DNAs as probes on either chromosome or resting nuclear preparations. The large cluster of hybridization signal in the centromeric and pericentromeric region produced by satellite I DNA probe appeared as a group of fluorescent sparks. This unique hybridization signal pattern also observed in resting nuclei. These findings suggested that the satellite I DNA chromatin is loosely packed with chromatin fiber extended out over a large area. Whereas, the hybridization signal with satellite II DNA appeared as pairs of distinct fluorescent spots located at the primary constrictions. These pairs of satellite DNA II signals also colocalized with the immunofluorescent signals produced by the human CREST anti-sera. These observations suggested that satellite II chromatin is more densely packed in an orderly manner. Such a way of chromatin packing may be a prerequisite for CENP binding, kinetochore reassembling, and the function of mammalian centromeres.

Nuclear Matrix Attachment Sequence is not Sufficient for in vivo Chromatin Loop Formation. *H.H. Heng, S. Bremer, G. Liu, W. Lu, J. Ye, S. Krawetz.* Center for Molecular Medicine & Genetics, Wayne State University, Detroit, MI.

Eukaryotic chromatin is thought to be organized as independent loops that can be visualized as a DNA halo. To form a loop domain, a certain proportion of chromatin must function as an anchor. Sequences associated with the nuclear matrix attachment region (MAR or SAR) have been proposed as providing this anchor function. To test this hypothesis, we established an experimental system of creating the in vivo condition of loop formation with defined MAR (SAR) sequences. By introducing known MAR fragments into mouse and human genomes by utilizing transgenic mice and cell transfection essays, we examined the inserted MAR sequence to determine if it is acting as an "anchor" sequence and if it is responsible for novel loop formation for both meiotic and mitotic loops. Our results of in situ loop digestion with DNase I as well as FISH detection on meiotic loops and mitotic halo clearly document that a percentage of integrated defined MAR sequences are tightly associated with the nuclear matrix of the host cell, serving as anchors for newly formed loops. This demonstrates the necessity of MAR for loop formation. However a large portion of these integrated MAR are detected as not associating with the nuclear matrix and therefore not serving as anchors. This demonstrates that not all MAR are used for anchoring loops, therefore MAR is not sufficient for in vivo chromatin loop formation. Clearly there is a regulation system of loop formation within cells to decide which MAR will be used as an anchor. Interestingly, we detected loop movement along the nuclear matrix. For the sequences tested, the attachment sites on the nuclear matrix were not fixed. This observation is not consistent with the model of fixed anchor sequences suggesting that anchor sites may switch between themselves. Further, if a new anchor is formed with non-MAR sequences, it is possible that other types of sequences can serve as anchors for loop formation as well. This dynamic anchor feature that we have discovered may be related to a novel regulatory transcription mechanism. In this paper, the detailed methodology and the novel features of chromatin loop domain are discussed.

A novel method for cloning and mapping of unusual sequences located in the human centromeric regions. *R. Sasi.*
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Most eukaryotic centromeres are composed of a strikingly complex array of varying types of tandemly repeating DNA elements. Normal human centromeres are enriched with alpha satellite DNAs, but the recently discovered human neocentromeres revealed that alphoid DNA is not a prerequisite for the centromeric activity. Sequencing of DNA from the Arabidopsis centromeres and human chromosome 21 centromeric regions indicated surprisingly the presence of varying classes of DNA elements that included expressed genes, pseudogenes and transposon like DNA sequences. Thus identifying those regions of human genome enriched with alpha satellites may not alone pinpoint regions that impart centromeric function. The traditional cloning and mapping strategies often failed to isolate all centromeric DNA sequences. In this report, we describe an efficient and new method for isolating those missed centromeric DNA elements. Flow- sorted human chromosome specific cosmid libraries were plated on agar plates and single colonies were picked up, inoculated individually in 96 well microtiter plates. Colonies were duplicated on to nylon membranes using 96 well replicators. These replicated membranes were hybridized at varying hybridization stringencies with human Cot-1 DNA, placental DNA and alpha satellites. Evaluation of the autoradiographic signal intensities enabled us to classify cosmids into three subgroups as strong, medium and weak intensities. FISH was performed to identify chromosomal locations of these cosmids. A substantial number of medium/weakly hybridizing cosmids were localized to the centromeric/pericentric regions of human chromosomes X, 8 and 17 (~34). Characterization of few of these centromeric cosmids by Southern hybridization, subcloning and sequencing confirmed the absence of alphoid sequences. Gamma satellite centromeric DNAs from chromosome X and 8 were successfully isolated using this approach. This method is useful for the systematic isolation of DNA sequences present in the human centromeric/pericentric regions. This newly isolated centromeric DNA may play a role in forming altered conformation that perhaps nucleate or stabilize normal centromeric structure.

A complex derivative chromosome 8 with an inverted duplication of 8p21.3@p23.3 and a rearranged duplication of 8q24.13@qter characterized by FISH. *Y.S. Fan^{1,2}, V.M. Siu²*. 1) Cytogenetics Lab, London Health Sciences Ctr, London, ON., Canada; 2) Department of Pediatrics, London Health Sciences Ctr and University of Western Ontario, London, ON., Canada.

We observed a complex derivative chromosome 8 in a newborn boy who had a low birth weight, multiple congenital anomalies and dysmorphic facies. The der(8) was further characterized at age 18 months by a high resolution G-banding analysis, spectral karyotyping, and FISH with multiple DNA probes. The karyotype was described to be: 46,XY,der(8)(qter@p23.3::p23.3@p21.3::q24.13@qter), representing an inverted duplication of region 8p21.3@p23.3 and a duplication of region 8q24.13@qter which was rearranged into the short arm. This young child has manifested a broad nasal bridge, micrognathia, cleft lip, hydrocephalus, partial corpus callosum agenesis, Dandy Walker malformation, congenital heart defects, dysplastic kidneys, hydronephrosis, marked hypotonia and significant psychomotor retardation. Some of these features are commonly seen in cases with an inv dup(8p) and in cases with a partial trisomy of 8q. It is known that a deletion in the terminal region of 8p was present in all the cases with an inv dup(8p) examined. In our case, however, such a deletion was not detected. Given that the duplication of region 8p22@p23.3 is not or less clinically significant, we have assumed that the phenotypic anomalies of this case was mainly the effects of trisomy 8q24.1@qter and trisomy 8p21.3@p22 which has been commonly involved in all the cases with an inv dup(8p).

Partial trisomy 18q due to a maternal insertion (2;18). *L. Campora*¹, *G. Zelaya*², *D. Montanari*², *J. Scheifer*², *C. Barreiro*², *M. Gallego*². 1) Clinical Genetics, CENAGEM, Capital Federal, Buenos Aires, Argentina; 2) Clinical Genetics, Hospital Garrahan, Capital Federal, Buenos Aires, Argentina.

Insertional or interstitial translocations are relatively rare chromosome aberrations (1/5000). They result from three chromosome breaks, the first two release an interstitial segment of chromosome which is then inserted into the gap created by the third break, without reciprocal exchange. We report a one year old girl, the only daughter of a healthy and non-consanguineous couple. The maternal obstetric history included a spontaneous abortion of first trimester. Pregnancy and delivery had been uneventful. No neonatal complications occurred. The girl had severe mental retardation, hypotonia and seizures without visual following and poor response at sounds. Physical examination demonstrated enlarged forehead, epicanthal folds, strabismus, low nasal bridge, small nose with bulbous tip, high arched palate, malformed ears, brachydactyly, mild interdigital webbing, hyperextensible joints, congenital heart disease and pale ocular fundus. Brain MRI showed slight cortical atrophy; EEG, occipital spikes, BAEP and VEP, increase of latency and echocardiogram, ventricular septal defect. Chromosome analysis of the proband revealed 46,XX,der(2)ins(2;18)(p23;q21.3 q11.2)mat. FISH using two colors painting probes for chromosomes 2 and 18 confirmed the G-banded interpretation. The father's karyotype was normal, and the mother was carrier of a translocation between chromosomes 2 and 18: 46,XX,ins(2;18)(p23;q21.3 q11.2). Trisomy 18q due to insertional translocations are rare and the present case is the third described. Two regions must work in conjunction, the proximal one (18q12.1 q21.2) and the distal one (18q22.3 qter), to produce the classical phenotype of trisomy 18. In addition, the duplication of 18q12.3 q22.1 may be associated with more severe mental retardation. The clinical findings in our patient support this conclusions.

FISH analysis of three cases of functional monosomy 10q26.3. *J.M. Gastier^{1,2}, B. Hamelberg¹, J. Labanowska¹, G.E. Herman³, D.W. Bartholomew^{1,3}, G.D. Wenger^{1,2}.* 1) Dept of Laboratory Med, Children's Hosp, Columbus; 2) Dept of Path, Ohio State Univ; 3) Children's Research Inst, Dept of Peds, Ohio State Univ.

Fewer than 30 cases of monosomy for distal 10q have been reported, and a consistent phenotype has not been described. We report the clinical findings in three new cases and describe FISH analysis used to confirm the karyotypes. Two of the cases have a terminal deletion [del(10)(q26.3)], while the other has translocation [t(X;10)(p22.3;q26.3)]. Chromosome 10 subtelomeric and chromosome Xp locus-specific FISH probes were utilized to characterize the breakpoints of the rearrangements. Case one is a five month old with failure to thrive, hypotonia, feeding intolerance, joint laxity, a prominent forehead, posteriorly rotated ears, but little facial dysmorphism. Cytogenetic analysis including FISH with chromosome 10 subtelomeric probes suggested the following karyotype: 46,XY,del(10)(q26.3).ish del(10)(q26.3)(tel 10p+,tel 10q-)de novo. Case two is a two year old referred for developmental delay, behavior problems, prominent ears and jaw, widow's peak, and no speech. Cytogenetic analysis revealed the karyotype 47,XYY,del(10)(q26.3).ish del(10)(q26.3)(tel 10q-). Case number 3 is a 16 month old female with developmental delay, occipital hair whorl, early hypotonia, no speech, staring episodes, sacral dimple, but little facial dysmorphism. Cytogenetic analysis suggested an abnormal 10q terminus. FISH analysis using chromosome 10 subtelomeric probes and probes for KAL and STS on Xp confirmed a translocation with the Xp breakpoint between STS and KAL [46,X,t(X;10)(p22.3;q26.3).ish t(X;10)(STS-,tel 10q+,KAL+;tel 10p+,tel 10q-,STS+,KAL-)de novo]. X inactivation studies revealed a random pattern in the patient's peripheral blood, suggesting that the phenotype may be due to inactivation of 10q26.3 sequences translocated to Xp. These cases contribute to the clinical characterization of a subtle cytogenetic abnormality in an attempt to better define a common phenotype. As subtelomeric FISH probes gain wider use, monosomy for chromosome 10q26.3 and small terminal deletions of other chromosomes may be observed more frequently, allowing for the report and definition of common clinical findings.

De novo 46,XY/47,XY,r(12) karyotype in a male with dysmorphia and psychomotor retardation. Review of the literature. V. Charpenel¹, A. Ion^{2,1}, L. Telvi¹. 1) Cytogenetique Constitutionnelle et Moleculaire, St Vincent De Paul Hospital, Paris, France; 2) Medical Genetics, St George's Hospital Medical School, London, UK.

We describe a male patient harbouring a de novo 46,XY/47,XY,+r(12) chromosome complement. The child presented with psychomotor retardation and dysmorphic face. Initially, standard karyotype analysis using R and G banding techniques showed the presence of a supernumerary marker chromosome in about 30% of the examined metaphases. This marker could not be identified by routine cytogenetic techniques, including high resolution and FISH using total painting probes for the most frequent chromosomes involved in the living new born trisomies (21, 18, 8 and X). However, a MULTIFISH analysis demonstrated the presence of three signals specific for the chromosome 12, suggesting that the marker was a ring r(12). Karyotype performed from peripheral blood in both parents showed a normal chromosome complement. At present, the frequency of supernumerary marker chromosomes has been found to be around 1/2500 in amniocenteses samples (Warburton, 1991) and mosaicism in association with a normal 46,XX or 46,XY cell line has been reported in about 70 % of the investigated cases. The present report emphasises the importance of updated cytogenetic and molecular techniques in identifying the origin of marker chromosomes. At present, only a few cases with supernumerary r(12) have been reported. They all had de novo mosaicisms, associated with important developmental delay and severe mental retardation. A review of to date published supernumerary r(12) reports is presented, as well as a phenotype/genotype correlation.

Deletion of candidate genes for AZF in an azoospermic man: confirmation by PRINS. *J.S. Kadandale¹, S.S.*

Wachtel², P.R. Martens¹, Y. Tunca¹, R.S. Wilroy¹, A.T. Tharapel¹. 1) Pediatrics, Univ Tennessee, Memphis, TN; 2) Ob-Gyn, Univ Tennessee, Memphis TN.

Among male patients evaluated for infertility, some 20% are affected with azoospermia or oligospermia. Cytogenetic investigations and molecular deletion analyses in these individuals have revealed that genes controlling spermatogenesis are located in the AZF (azoospermia factor) region at interval 6 of the Y chromosome, corresponding to sub-bands Yq11.22-q11.23. Microdeletions have been observed within the AZF region in some of these patients. Using a dual approach involving PRINS (primed in situ labeling) and conventional FISH, we studied a 27-year-old man referred for evaluation of azoospermia. Initial analysis of cultured blood lymphocytes revealed a 45,X/46,X,+mar karyotype, but QFQ-banding failed to identify a fluorescent area on the marker chromosome. On analysis with FISH, the marker was found reactive with probes AMELY and DYZ3, confirming that it was Y-derived. Subsequent FISH with dual color whole chromosome paint probes for X and Y chromosomes showed an Xp-Yp interchange. By PRINS, we then identified translocation of the SRY gene from its original location on Yp, to Xp, thus confirming the derivative nature of the X chromosome. PRINS studies with primers for RBM and DAZ, the two candidate genes for AZF, confirmed deletion of these genes in the patient but not in unaffected males. These observations resulted in a complex karyotype: 45,der(X),t(X;Y)(p22.3;p11.2)/46,der(X)t(X;Y)(p22.3;p11.2).prins der(X)(p22.3)(SRY+),del(Y)(q11.22q11.23)(RBM-,DAZ-). Loss of the der(Y) in about 8% of the cells during mitosis is a common characteristic of ring chromosomes. The formation of a ring in this instance could be due to the Xp-Yp interchange with subsequent deletion of the Y long arm, subsuming the AZF region. Detailed investigation of this patient, with PRINS, enabled us accurately to define the abnormality, and to postulate that azoospermia in this case had resulted from deletion of the RBM and DAZ genes. We conclude that PRINS is a useful method for the evaluation of male infertility, especially in cases of azoospermia.

Directly Defining the Gene(s) for Genomic Disease: Use of the Sequence-Integrated BAC Resource to analyze a subtle deletion/inversion involving chromosome 8p22-23.3. *Q-J. Liu¹, X-N. Chen¹, S. Gersen², C. McKee², M. Hummel³, S. Graham³, J.R. Korenberg¹, G. Sun².* 1) Medical Genetics Birth Defects Center, Cedars-Sinai Medical Center, Los Angeles, CA; 2) DIANON Systems, Inc. Stratford, CT; 3) Department of Pediatrics, West Virginia University, Morgantown, WV.

Cytogenetic aberrations of the distal short arm of chromosome 8 characterize a number of clinically defined disorders. However, neither the genotype/phenotype correlation nor the genomic structures that predispose to the defects are yet delineated.

We report the clinical & molecular analysis of a 23-year-old woman with mental retardation, seizures and scoliosis, all of which are common features reported in cases of 8p rearrangement. Cytogenetics and FISH studies with an 8p telomeric probe showed a deletion of the terminal and an inversion with a possible duplication involving 8p23. Parental chromosomes are normal. Using our Integrated BAC Resource (Korenberg *et al.* 1999), high-resolution multi-color FISH analysis was performed on metaphase and interphase cells. Ten BAC DNA probes mapping on 8p22 and 8p23 were tested including 5 BACs carrying unspecified locally repeated sequences. The results revealed: 1) B366 on 8p23.3 was deleted confirming routine cytogenetics; 2) B26G3 on 8p23.2 carrying repeated sequences detected a subtle partial deletion; 3) B1113 on 8p22 carrying repeated sequences was not only partially deleted but also involved in an inversion. No clear duplications were shown. The analysis of this case has gone beyond the simple genomic DNA sequence and demonstrates the ability to detect and study the subtle reorganization of the genomic material using the Integrated BAC Resource. By linking these results with gene, genetic and physical maps (STS, end sequence & fingerprint), we can move towards identification of genes that may be responsible for the phenotypes seen in this patient. In the current case, we have demonstrated that the phenotypes of mental retardation and scoliosis may be caused by haploinsufficiency rather than duplication of the gene(s).

Clinical application of sequence-based single copy probes for FISH. *J.H.M. Knoll^{1,2}, P. Cazcarro¹, P.K. Rogan^{1,2}.*
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Commercial probes currently exist for the most common genomic rearrangements, however, these probes often do not permit analysis of rare breakpoints or less common aneuploidies. We have developed a technique that provides custom single copy probes for chromosome analysis (scFISH). scFISH relies on the availability of previously determined sequences in known disease intervals, and the ability to deduce the locations of single copy sequences in these intervals.

Typically, a genomic sequence of 100-300 kb was scanned to identify the precise locations of all repetitive sequence family members. The longest intervening single copy intervals were identified and amplified by long PCR. The purified amplicons were then labeled and hybridized by standard FISH procedures, except that suppression of repetitive DNA hybridization was unnecessary.

Specimens from patients with Prader-Willi, Angelman, duplication chromosome 15, DiGeorge and monosomy 1p36 syndromes were analyzed with probes developed adjacent to NDN (15q12), and from HIRA (22q11.2) and CDC2L1 (1p36.3) genomic sequences, respectively. The NDN probes consisted of three fragments, 4166 bp (70 kb downstream of NDN), 3544 bp (48 kb downstream) and 2290 bp (44 kb downstream) derived from GenBank accession AC006596. The HIRA probes were composed of 4 fragments, 5170 bp (IVS12 to IVS13), 3691 bp (IVS21 to IVS24), 3344 bp (IVS13 to IVS15) and 2848 bp (IVS2 to IVS4) from GenBank accession NT_001039. The CDC2L1 probes comprised two fragments, 4823 bp (3' UTR) and 4724 bp (IVS11 to 3' UTR) from GenBank accession AL031282. These probes were used individually or in combination to detect sequences in either metaphase or interphase cells and to examine allele-specific replication. These probes detected the expected abnormality in each patient specimen analyzed. Detection of other contiguous gene syndromes and common translocations in hematopoietic malignancies will also be presented.

Severity of developmental delay is associated with the proportion of cells with functional X disomy in female patients with mosaic for small ring X chromosomes. *T. Kubota¹, K. Wakui¹, Y. Watanabe², M. Yoshino², N.*

Okamoto³, T. Kida⁴, H. Ohashi⁵, Y. Fukushima¹. 1) Dept of Clinical and Molecular Genetics, Shinshu Univ School of Med, Matsumoto; 2) Dept of Pediatrics, Kurume Univ School of Med, Fukuoka; 3) Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka; 4) Div of Pediatrics, Hakodate Central Hospital, Hokkaido; 5) Div of Medical Genetics, Saitama Children's Med Ctr, Saitama, Japan.

Developmental delay (DD) not usually associated with Turner syndrome is seen in some females with mosaic for small ring X chromosomes [45,X/46,X,r(X)]. However, mechanisms of DD in these patients are not well understood. We have performed quantitative HUMARA X-inactivation assay by methylation-specific PCR using an automated sequencer. Of the 4 female patients analyzed, two patients with severe DD [46,X,r(X) lines are 73%, 87%, respectively] showed both maternal and paternal unmethylated (U) alleles derived from the active X and did not show methylated (M) alleles derived from the inactive X, suggesting that both normal X [nl(X)] and r(X) are active in all 46,X,r(X) cells (complete functional X disomy: CFXD). One patient with moderate DD showed both maternal and paternal U-alleles and one maternal M-allele, suggesting that both nl(X) and r(X) are active in some proportion of cells (~40%). The remaining patient with mild DD showed an U-allele and a M-allele (different allele size), suggesting that a nl(X) is active and an r(X) is inactive in all cells. These results suggest that the severity of DD may be associated with the proportion of cells with the functional X disomy in female patients with a small r(X). XIST locus on an r(X) was positive in three patients by FISH, but was negative in one patient with severe DD, in whom CFXD is likely to be caused by lack of the XIST locus. However, the remaining patient with severe DD preserved the XIST locus on an r(X), and did not show uniparental X disomy, suggesting no somatic duplication of an X chromosome. Therefore, CFXD in this patient may be caused by either defect of the XIST gene function or impairment of the putative X-chromosome counting process which both nl(X) and r(X) are counted to be active.

The predictive value of FISH analyses for possible occurrence of Wilms tumor in aniridia patients with/without chromosome abnormalities around 11p13. *R. Muto*^{1,3}, *S. Yamamori*², *M. Osawa*¹, *H. Ohashi*³. 1) Department of Pediatrics, Tokyo Womens Med. Univ., Shinjuku-ku, Tokyo Japan; 2) Research department Mitsubishi Kagaku Bio-Clinical Laboratories, Inc.Tokyo Japan; 3) Division of Medical Genetics, Saitama Children's Medical Center, Saitama Japan.

Aniridia is an autosomal dominant condition caused by haploinsufficiency of the PAX6 gene at 11p13. The abnormalities of the PAX6 gene include mutations in the gene, position effect and gross deletions (cytogenetically visible or non-visible) around the gene. When gross deletions involve adjacent genes including WT1 (about 750 kb from PAX6), patients are predisposed to develop Wilms tumor and associated abnormalities (WAGR syndrome). We studied 17 patients with aniridia (10 are chromosomally normal and 7 abnormal) for possible deletion around 11p13 by FISH with four probes: PAX6, D11S2163 ,PER and WT1. Eight patients had gross deletions spanning the entire four loci, among whom 5 developed Wilms tumor, while none of the other 9 patients without detectable deletion have developed Wilms tumor. Four papers have been previously published to study microdeletions in the 11p13 region by means of molecular methods in aniridia patients. Out of a total of 101 patients in these five reports including this report, 29 patients had a deletion spanning from PAX6 to WT1 loci, of whom 14 developed Wilms tumor (48.3%). None of the other patients without deletion developed Wilms tumor. Risk assessment of Wilms tumor occurrence in aniridia patients by fine evaluation around the 11p13 region using FISH would be reliable and practical.

Two cases of 46,XX SRY positive males: Unexpected results on molecular analysis of Y sequences with a molecular deletion detection screening panel. *E. Mak-Tam, D.J. Allingham-Hawkins, A. Angelo, G. Rasiuk, J. Tzountzouris, P.R. Wyatt.* Genetics, North York General Hosp, Toronto, Ontario, Canada.

46,XX males occur with a frequency of about 1/20,000 births. The phenotype is heterogeneous ranging from normal male to genital ambiguity, but all have testicular development and are infertile. This variation is largely due to the genetic heterogeneity among the XX males. They can be classed as SRY, (sex determining region Y) positive, negative or true hermaphrodite. About 80% of XX males are positive for the SRY gene, which has been mapped to Yp11.3. The most common mechanism for XX male (and XY female) formation involves an aberrant X-Y exchange during paternal meiosis. Two cases were referred for infertility and hypogonadism respectively. The derivative X was not detected with routine G-Banding (500 band level), but FISH identified the presence of SRY in both cases. Molecular analysis using the Promega™ Y deletion detection screening panel for 18 sequence tag sites (STS) covering the four AZF regions was used to determine the extent of the translocated Y sequences. Analysis of case 1 not only showed positive for the SRY and ZFY (zinc finger protein, Y-linked) loci, but also an unexpected Yq locus AZFc (azoospermia factor c), distal to a deletion gap. This may indicate a more complex interchange or an inverted Y origin. The clinical, FISH and molecular data for the two cases will be presented. The increased availability of screening tools allows for more accurate delineation of rearrangements. In addition, clinical labs can provide more informative testing for referrals such as ambiguous genitalia.

Chromosomal rearrangements identified by M-FISH, FISH and subtelomeric probes. *C.B. Lozzio¹, E.*

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Two cases with marker chromosomes identified by M-FISH and one case with a subtelomeric deletion of the long arm of chromosome 2 are reported. M-FISH studies were performed using the Multicolor Spectra Vysion Assay from Vysis and the Applied Imaging Cytovision version 4.1 for UNIX with software for M-FISH. The subtelomeric deletion was detected with the Chromoprobe Multiprobe-T system from Cytocell. A mosaic for a small marker chromosome was found to be derived from chromosome 5 by M-FISH and this was confirmed by FISH studies with whole chromosome and centromeric probes for this chromosome. The child is a 2 years old non-dysmorphic male with developmental delay, tall stature and microcephaly. The finding that the extra marker is derived from chromosome 5 indicates that he has partial trisomy for the pericentric region of this chromosome. Another mosaic for a small marker was observed in a 14 years old female with short stature, menarche at 10 years and precocious adrenarche. She has a pericentric inversion of one of the X chromosomes and FISH studies showed that the X inactivation region XIST(Xq13) has moved to the short arm in the inverted X chromosome. The M-FISH studies revealed that the small extra marker is derived from chromosome 7 and FISH studies confirmed that this marker contains the centromere of chromosome 7. Thus, this child has partial trisomy for the pericentric region of chromosome 7. A cryptic subtelomeric deletion of the long arm of chromosome 2 was detected by the Multiprobe-T from Cytocell in a premature infant with multiple dysmorphic features, pulmonary hypoplasia and maternal history of polyhydramnios. Metaphase chromosomes in the amniotic cells cultures and in cultures of blood were normal at a 450 band level. These cases are examples of clinical applications of new molecular cytogenetic techniques that allow the identification and characterization of chromosomal rearrangements of clinical significance.

Rx FISH in conjunction with G banding: One step solution to resolve complex denovo marker chromosomes and intrachromosomal rearrangements. *S.P Naber, M. Karamove, C. Phillips, A. Nasir, R. Naeem.* Department of Pathology, Baystate Medical Center, Tufts University School of Medicine, Springfield MA.

In addition to SKY FISH and M FISH one of the recent advances in FISH technology is Rx FISH or multicolor multiband chromosome analysis. SKY and M FISH are reasonable approaches for identifying marker chromosomes but have limitations in identifying intrachromosomal changes such as inversions and insertions. In conjunction with G banded analysis, Rx FISH can be a useful tool to identify marker chromosomes and intrachromosomal rearrangements. We present two cases where we used G banding, routine FISH, and Rx FISH to identify a de novo complex chromosomal rearrangement and an intrachromosomal paracentric inversion insertion. The first case was a bone marrow sample from acute non-lymphoblastic leukemia with multiple translocations and a derivative chromosome. G banded analysis not able to identify and confirm all the chromosome aberrations; therefore routine FISH and Rx FISH were applied to resolve these rearrangements. The final karyotype was 45,XX, t (8; 12)(q12; p13), -15, der (17) t (15; 17)(q23; q22). The second case was a familial chromosomal abnormality with multiple family members inheriting the same marker 17 chromosomes. In addition to G banding and routine FISH, Rx FISH was applied to resolve this paracentric inversion insertion. G banded chromosome analysis has limitations in resolving marker chromosomes. Rx FISH, in conjunction with routine cytogenetics, proved to be a simple one-step solution for resolving complex as well as intrachromosomal rearrangements.

Sex identification from bloodstains of normal persons and sex reverse cases using FISH and PCR. *S.M. Tayel¹, S.J. Abulhasan²*. 1) Genetics Unit, Anatomy Dep., Alex - Faculty of Medicine , Alexandria , Al Azarita, Egypt; 2) Kuwait Medical Genetics Center - Maternity Hospital, Kuwait.

Sex identification of dry blood and decomposed tissues is of crucial importance both in clinical and forensic medicine. Sixty phenotypically normal males (36) and females (24), and 7 cases of sex reverse; 3 phenotypic females with Swyer syndrome and the 46, XY karyotype, and 4 phenotypic Klinefelter's-like males with the 46, XX karyotype were subjected to sex identification by FISH and PCR using bloodstains. Sex identification using X/Y cocktail probe (DXZ1 & DYZ1, Oncor) and FISH has correctly identified the male sex in 91.69% of the interphase nuclei of the 36 males of the study, and in 92.29% of the cells of the 24 females and incorrectly identified the 3 females with Swyer syndrome as males and the 4 Klinefelter's-like males as females. The 60 normal persons of the study was correctly typed to their phenotypic sex by the 2 PCR methods used i.e. the single PCR reaction using the amelogenin sequence specific for the X and Y chromosomes and the multiplex PCR using SRY gene (male-specific) and the AR gene (X-specific). Out of the 7 cases with sex reverse, one Klinefelter's-like male was incorrectly identified as female due to absence of amplification of the SRY gene and the amelogenin male-specific 788 bp fragment. The present study demonstrates that both FISH and PCR techniques are fast, easy to perform, reliable and efficient for sex identification but PCR is more accurate. It also stresses that the sex identified is the genotypic sex which does not necessarily corresponds to the phenotypic one and in cases of doubt, sex reverse has to be thought-off and ruled out using different X-and Y-specific probes and PCR.

Clinical and molecular-cytogenetic studies in seven patients with ring chromosome 18. *P. Stankiewicz¹, I. Brozek², Z. Hélias-Rodzewicz¹, J. Wierzbą³, J. Pilch⁴, E. Bocian¹, A. Balcerska³, A. Wozniak², I. Kardas², J. Wirth⁵, T. Mazurczak¹, J. Limon².* 1) Department of Medical Genetic, Institute of Mother and Child, Warsaw, Poland; 2) Department of Biology and Genetics; 3) Department of Pediatrics, Medical University, Gdansk, Poland; 4) Department of Pediatric Neurology, Silesian School of Medicine, Katowice, Poland; 5) Roper's Department, Max-Planck-Institute for Molecular Genetics, Berlin, Germany.

We report the results of detailed clinical and molecular-cytogenetic studies in seven patients with ring chromosome 18. Classical cytogenetics and FISH analysis with chromosome 18 painting probes identified five non-mosaic and two complex mosaic 46,XX,dup(18)(p11.2)/47,XX,dup(18)(p11.2),+r(18) and 46,XX,dup(18)(p11.32)/47,XX,dup(18)(p11.32),+r(18) karyotypes. FISH analysis was performed for precise characterization of the chromosome eighteen breakpoints using chromosome 18 specific short arm paint, centromeric, subtelomeric and a panel of fifteen Alu- and DOP-PCR YAC probes. The breakpoints were assessed with an average resolution of ~2.2 Mb. In all r(18) chromosomes the 18q terminal deletions ranging from 18q21.2 to 18q22.3 (~35 and ~9 Mb, respectively) were found whereas only in four cases the loss of 18p material could be demonstrated. In two patients the dup(18) chromosomes were identified as inv dup(18)(qterp11.32::q21.3qter) and inv dup(18)(qterp11.32::p11.32p11.1::q21.3qter)pat, respectively, with no evidence of 18p deletion. A novel inter-intrachromatid mechanism of formation of duplications and ring chromosomes is proposed. Although the effect of ring instability syndrome cannot be excluded, the phenotypes of our patients with characteristic features of 18q- and 18p- syndromes are compared and correlated with the analyzed genotypes. It has been observed that a short neck with absence of cardiac anomalies may be related to the deletion of the 18p material from r(18) chromosome.

FISH as an initial screen for aneuploidy in spontaneous abortion specimens. *A.J. Sobrino¹, J.K. Kline^{2,3}, D. Warburton^{1,3}.* 1) Genetics Laboratory, New York Presbyterian Hospital, New York, NY; 2) New York State Psychiatric Institute, New York, NY; 3) Columbia University, New York, NY.

Culture of specimens from spontaneous abortion specimens has inherent problems that can lead to failure to obtain the fetal karyotype: lack of growth of in culture, contamination of the culture by microorganisms or overgrowth of maternal cells from the specimen. Interphase FISH on enzyme-digested fetal tissues before culture is one method of avoiding these problems. While a complete aneuploidy scan is theoretically possible, we have opted for a multiplex system that allows detection of aneuploidy for chromosomes 13,15,16,18,21,22, X and Y. This will diagnose not only trisomy for these chromosomes, but also triploidy, monosomy X and tetraploidy. Together these comprise 80% of the chromosome abnormalities in spontaneous abortions, and about 40% of early losses. Only those specimens in which aneuploidy is not detected by FISH need to be continued in culture.

Comparison of the results of this approach in our laboratory to standard cultures indicates that it is not only rapid but cost effective, since it avoided culturing in 42% of cases. It also enabled a diagnosis of aneuploidy in several cases where the culture failed to grow, or where the karyotype would have been read as normal female because of maternal cell growth.

Increased nondisjunction in sperm identified as 'normal' by strict morphology in infertile patients: Implication for ICSI(intracytoplasmic sperm injection). *H.M. Ryu^{1,2}, W.W. Lin³, D.L. Lamb³, W. Chuang³, L.I. Lipshultz³, F.Z. Bischoff²*. 1) Dept OB/GYN, Samsung Cheil Hos, Sungkyunkwan Univ, Seoul, Korea; 2) Dept OB/GYN, Baylor College of Medicine, Houston,TX; 3) Dept Urology, Baylor College of Medicine, Houston, TX.

As part of the ICSI procedure, normal sperm are selected on the basis of motility and morphology. Although the incidence of aneuploidy among sperm with abnormal morphology has been evaluated, little is known regarding the genetic integrity of normal appearing spermatozoa among patients identified as infertile based on abnormal strict morphology analysis. The objective of this study was to determine the incidence of nondisjunction in morphologically normal sperm of infertile men who are candidates for ICSI. Method: Semen smears from eight men undergoing ICSI with abnormal strict morphology (<4% by Kruger's criteria) were obtained. For controls, semen smears from normal fertile men (n=6) were studied. Following standard hematoxyline staining, sperm with normal morphology were identified using Kruger's strict morphology criteria. In each case, the locations of 100-150 morphologically normal appearing sperm were recorded using an electronic microlocator. Slides were subsequently subjected to FISH for detection of chromosomes X, Y, and 18. Nuclei were then relocated and analyzed under the fluorescent microscope. Ploidy of each cell was determined according to the number of signals detected for each chromosome-specific probe. Results: Among the infertile group, aneuploidy was observed in 1.8 - 5.5% of the morphologically normal sperm as compared to 0 - 2.6% among the fertile control group. Statistically significant differences ($p=0.004$) in the incidence of chromosome aneuploidy was observed. Conclusion: These results may help explain why some pregnancies fail among ICSI patients. Moreover, the results demonstrate that normal morphology is not an absolute indicator for the selection of genetically normal sperm. Therefore, ICSI patients need to be counseled that although morphologically normal sperm are being selected, there is still some risk for having a genetically abnormal offspring.

Cryptic translocation 10pter;18qter as a cause of two different microdeletions in the offspring. *M.S. Somer¹, P. Tienari², M. Kähkönen³*. 1) Medical Genetics, Family Federation of Finland, Helsinki; 2) Department of Neurology, Helsinki University; 3) Department of Clinical Genetics, Tampere University Hospital, Tampere, Finland.

The terminal 18q deletion is characterized by mild to moderate mental retardation, hypotonia, delayed CNS myelination, low IgA levels, susceptibility to autoimmune disorders, and typical dysmorphic features. We report a three-generation family with 5 males and 4 females with different grades of psychomotor retardation. Three of them had rheumatoid arthritis, two IgA deficiency, and one diabetes mellitus. Prometaphase study with resolution of 850 bands showed no abnormality, although deletion of 18qter was specifically looked for. Brain MRI of two patients showed severely delayed myelination at ages 8 months and 4 years, respectively. Myelin basic protein (MBP) gene and its neighboring polymorphic markers were studied in one and shown to be deleted based on segregation analysis. Finally, a telomeric deletion of 18q was verified with TelVysion 18q telomere probe in five of the six patients available for study. In healthy carriers of the family, this probe was shown to hybridize to terminal 10p, whereas the TelVysion 10p telomere probe hybridized to terminal 18q. One affected family member with a distinctively different phenotype and normal brain myelination was shown to have the counterpart translocation product with deletion of the telomeric 10p and trisomy of telomeric 18q region. It is now often possible to verify a clinically suspected chromosomal disorder with FISH. Two different mental retardation syndrome phenotypes within one family may be explained by different unbalanced karyotypes derived by segregation of a parental cryptic translocation.

Chromosome analysis on fetal tissues: A revised protocol employing interphase FISH improves the success rate.

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Our laboratory receives many requests for cytogenetic studies of tissues from SABs, some of which are autolyzed, precluding traditional metaphase chromosome analysis. In 1996 we revised our solid tissue protocol to include preparation of cells for possible FISH analysis. Fresh cells are saved in 3:1 methanol:acetic acid following disaggregation in 1ug/ml collagenase Type1A (Sigma #C9891) and 75 mM KCl treatment. In addition FISH studies can be pursued using paraffin-embedded tissues in cases in which fresh fixed cells were not prepared. Using these methods, 74 cases were tested including 30 with no growth, 19 with incomplete cytogenetic analysis, and 25 paraffin-embedded tissue samples. DNA probes (LSI13, D15Z, D16Z3, D18Z1, LSI21, DXZ1, DYZ1 and SRY, Vysis, Inc., Downers Grove, IL) were selected based on ultrasound or autopsy findings, and in cases with incomplete karyotypic analyses according to the chromosome(s) in question. FISH results were obtained in 28/30 (93%) of the no growth cases. In 13/28 (46%) cases, aneuploidies were revealed including four cases of trisomy 18, four of monosomy X, two of trisomy 21, one triploid and one each of trisomies 13 and 16. In 2/30 (7%) cases with no FISH result severe maceration may be a factor. Among 19 cases with incomplete karyotypic analyses, suspected chromosome abnormalities were found in six, the origin of a marker chromosome was identified in one, and in another case, a female karyotype (46,XX) was found in skin cells from a fetus with ambiguous genitalia. In the remaining 11 cases, FISH revealed disomy for the chromosome(s) in question. FISH results were obtained in 23/25 (92%) cases of paraffin-embedded fetal tissue. In 10/23 (44%) cases, aneuploidies were detected including three cases of trisomy 21, two of trisomy 13, two with monosomy X, two triploids and one case of trisomy 16. No FISH result was obtained in 2/25 (8%) cases.

Our results indicate that interphase FISH is an important supplement to routine karyotypic analysis of SABs and can provide valuable information for genetic counseling.

Breakpoint mapping of an apparently balanced 2;8 translocation reveals a cryptic deletion on 2q in a child with autism. *I. Borg^{1,2}, K. Stout³, D.R. Sargan¹, D. Morgan⁴, L. Willatt⁴, V. Kalscheuer³, N. Tommerup⁵, H.H. Ropers³, M.A. Ferguson-Smith¹.* 1) Department of Clinical Veterinary Medicine, University of Cambridge, Cambridge, UK; 2) Medical Genetics Department, Addenbrooke's NHS Trust, Cambridge, UK; 3) Max-Planck Institute for Molecular Genetics, Berlin, Germany; 4) Cytogenetics Lab, Addenbrooke's NHS Trust, Cambridge, UK; 5) Department of Medical Genetics, IMBG, Copenhagen, Denmark.

Autism is a neurodevelopmental disorder characterised by abnormalities in social, communicative and behavioural functioning. The aetiology and the evidence suggest that the risks are likely to involve non-genetic, as well as genetic factors; the latter have probably the most powerful influence. The occurrence of chromosome abnormalities has been reported in association with a clinical phenotype of autism. Two International Consortia conducting a genome-wide scan of autism reported 2q as one of the potential susceptible regions in the search for candidate genes for autism. We report on a de novo balanced translocation t(2;8)(q36; q21), in a male child with delayed developmental milestones and marked impairment in communication and social interaction. Repetitive, stereotyped activities and behaviour, formed part of the clinical picture. Minor dysmorphic features included upward slanting palpebral fissures and prominent antihelices. Several molecular cytogenetic techniques were employed in the search for cryptic chromosome abnormalities. YAC clones in the region of the breakpoint were selected and used as FISH probes; one of these was found to be deleted on 2q. Further YAC FISH is in progress to delineate the extent of the sub-microscopic deletion. Likewise we expect to narrow the search for autism susceptibility in the region of interest on 2q. The results present a step towards identifying genes predisposing to autism.

Double Aneuploidy:Down's syndrome associated with Klinefelter's syndrome. *F.A. Ali, S.S. Al Arrayed, H. Sanad, A. Al Abbasi.* Genetic department, Salmaniya Medical Complex, Manama, Manama, Bahrain.

The occurrence of double aneuploidy, i.e. the existence of two meiotic non-disjunction events is relatively rare. Although the association between double autosomal trisomy, such as trisomy 21&18 is extremely rare, rare cases of combination between Down syndrome and gonosomal trisomy has been reported. 8 year old Indian boy with Down and klinefelter syndrome is reported. The patient condition resulted from de novo trisomy-21 with extra X-chromosome (48,XXY,+21). The patient was born normally with birth weight, length and head circumference of 2590 gram, 45 cm and 31 cm respectively. The age of mother and father were 31 and 32 years old at the time of delivery. He had dysmorphic features of Down syndrome with imperforated anus, severe mental retardation, small phallus and bilateral undescended testicles but no congenital heart disease. The weight and hight were on the 3rd centile while the head circumference below the third centile. The patient developed hypothyroidism by the age of 6 years and was maintained on L-thyroxin. Testosterone level was pre-pubertal and failed to rise after HCG stimulation test.

Molecular cytogenetic investigation of a supernumerary marker reveals a cryptic translocation in a prenatal sample. W.L. Golden¹, T.E. Kelly¹, S. Thiagarajah², J. Boner², K.W. Sudduth¹, S.H. Burnett¹, A.W. Lambert¹, C. Jackson-Cook³. 1) Department of Pediatrics, Univ of Virginia Health Sciences Ctr, Charlottesville, VA; 2) Martha Jefferson Hospital, Charlottesville, VA; 3) Department of Genetics, Medical College of Virginia, Richmond, VA.

A 36 year old prima gravida referred for prenatal diagnosis due to advanced maternal age and a MSAFP triple screen risk of 1/57 for Down syndrome underwent an amniocentesis at 22 weeks gestation. Standard GTG cytogenetic analysis revealed that the majority of cells (33/40) contained a small, supernumerary marker chromosome. Molecular cytogenetic analysis using SKY technology revealed that the marker originated from a chromosome 2. Subsequent whole chromosome paint and alpha-satellite analysis confirmed this finding. SKY analysis also revealed that there appeared to be a small amount of chromosome 12 material present on the short arm of one of the number 2 chromosomes. By SKY analysis there was no evidence of the reciprocal chromosome 2 material being present on either chromosome 12s. However, whole chromosome paints for 2 and 12 confirmed the finding of a portion of 12 on the short arm of 2 and revealed a small portion of chromosome 2 material on the long arm of chromosome 12. Even in retrospect this rearrangement is not apparent on standard GTG analysis. Standard and molecular cytogenetic analysis on the parents revealed that neither carried the t(2;12), however, the father was found on GTG analysis to have 3/200 cells with a supernumerary marker that appeared similar to that seen in the fetus. Counseling issues included the *de novo* nature of the t(2;12) as well as the significance of the frequency and distribution of what may be a familial marker. The couple opted to continue the pregnancy and at 39 1/2 weeks gestation an apparently normal female infant was delivered. Birthweight was 2807 gm with Apgars of 8 and 9. This case illustrates both the power and limitations of the methodologies available to the cytogeneticist for prenatal cases.

The significance of a prenatally diagnosed del(10)(q23). *J.E. Fox¹, A.L. Zaslav², S. Allan¹, R. Kazi², T. Shklooskaya³, D. Sohal³, S.M. Kleyman³, R.S. Verma³.* 1) Human Gen., Schneider Child Hosp, Long Island Jewish Medical Ctr, New Hyde Park, NY; 2) Dept of Pathology, Long Island Jewish Medical Center, The Long Island Campus of the Albert Einstein College of Medicine, New Hyde Park, NY; 3) Wyckoff Heights Medical Center, Brooklyn, NY, and New York Hospital/Weill Medical College of Cornell University, New York, NY.

The expression of fragile sites is highly variable and information is limited. We report a case of prenatal mosaicism for a deletion of chromosome 10(q23). It was suspected that this abnormality resulted from an inherited fragile site. In situ amniocentesis analysis was performed on a 27-year-old, East Indian female for an abnormal MSAFP. The karyotype was 46,XY,del(10)(q23)[9]/46,XY[45]. Chromosome analysis of parental blood grown in standard and in low-folate medium demonstrated the presence of the fragile site in 2/138 cells (standard) and in 6/50 cells (low-folate) in the mother. A repeat amniocentesis revealed 46,XY in 100 cells. A high-resolution ultrasound and fetal echocardiogram were performed at 22 weeks and neither revealed abnormalities. There are only three reports of prenatally diagnosed cases of del(10)(q23). Two cases apparently resulted from an inherited fragile site (Hsu, et al., 1996). One resulted in a normal live born. The other pregnancy was interrupted and the fetus appeared normal. The third case also resulted in a normal live born although no parental fragile site was detected. It appears that prenatally diagnosed mosaicism for a del(10q) represents culture artifact and is not clinically significant. Based on these findings the patient elected to continue the pregnancy. Since fragile site expressivity is variable and data is scarce, counseling should include the following recommendations: (1) cytogenetic analysis of parental blood grown in appropriate induction medium, (2) high-resolution ultrasound and fetal echocardiogram, and (3) postnatal confirmatory studies. Additional cases of prenatally diagnosed mosaicism of deletions are necessary to confirm the relevance of these findings.

Familial pericentric inversion of chromosome 5 in a family with benign neonatal convulsion. *D. Concolino¹, M. Iembo¹, M. Moricca¹, E. Rossi², O. Zuffardi², P. Strisciuglio¹.* 1) Department of Pediatrics, Faculty of Medicine University of Catanzaro ITALY; 2) Department of Biology and Medical Genetics University of Pavia ITALY.

The BFNC is an idiopathic autosomal dominant form of epilepsy characterized by spontaneous partial or generalized clonic convulsions beginning within the first months of life. A family is described in which the grandmother, the mother and the patient presented "benign" neonatal convulsions. The proband, a male, was a second child of unrelated parents, born at 40 weeks of gestation after an uneventful pregnancy. Apgar scores were 10 at 1 and 5 minutes. At delivery the weight, the length, the head circumference and the clinical examination were normal. In the third day of life he had five episodes of generalized clonic convulsions treated with phenobarbital (5mg/Kg/day). Routine laboratory and neurologic investigations were normal. At 8 months of age he was developmentally normal and continued to take phenobarbital. The mother and maternal grandmother appeared phenotypically and intellectually normal but reported a clear history of convulsions in neonatal age and afebrile seizures later in childhood. Chromosome analysis for proband and his family were performed from synchronized peripheral lymphocyte cultures. The proband's karyotype was interpreted as 46, XY, inv (5)(p15-q11.), the mother appeared to have the same rearrangement of chromosome 5. Moreover in the mother's family, the grandmother was carrier of inversion while other relatives had normal karyotypes. FISH experiments were performed to locate the breakpoints on chromosome 5 [inv5(p15.1,q11.2)]. In this report we describe a family carrying a pericentric inversion of chromosome 5 (p15.1,q11.2) presenting benign familial neonatal convulsions (BFNC). Loci for BFNC, based on the linkage analysis, have been mapped to human chromosomes 20q13.3 and 8q24 and recently a potassium channel gene, located on 20q13.3 was isolated and its mutations supposed to be responsible for BFNC. This report suggests the possibility of a new locus for BFNC on the chromosome 5 and confirms the genetic heterogeneity of this disorder.

A de novo duplication of marker chromosome inv dup(15). *Y.H. Cho¹, C.H. Lee¹, S.H. Shim¹, Y.J. Park², H.C. Kwon², W.I. Park², H.J. Lee².* 1) Dept Medical Genetics, Hanyang Univ Col Medicine, Seoul 133-791, Korea; 2) Department of Obstetrics and Gynecology, School of Medicine, Eulji University, Seoul 139-711, Korea.

During the cytogenetic study of a 40 years old woman referred for the evaluation of missed abortions, two identical marker chromosomes were detected (48, XX, +marx2). The markers were further evaluated with C-band and NOR-stain and her parents were karyotyped. The overall length of the marker chromosome was smaller than chromosome 21 and looked like metacentric chromosome. There was no G (+) band on both arms, and satellites were detected on both arms on NOR stain. Her father had one copy of the same marker chromosome (47, XY, +mar). The origin of the marker chromosome was evaluated by FISH. With D15Z1 probe (15p11.2) green signal was detected on both arms of the marker chromosomes. So the marker chromosomes were thought to be common harmless inv dup (15). The missed abortion history of the patient was thought to be unrelated with the marker chromosomes. This case may be the first report on the de novo duplication of inv dup (15).

A maternal complex chromosome rearrangement ascertained through a mildly affected child with a del 13q. L.M.

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Carriers of complex constitutional chromosome rearrangements (CCRs) are usually ascertained after a the diagnosis of an unbalanced karyotype in malformed offspring, or if chromosome studies are done due to repeated pregnancy losses.

We report a child with an interstitial deletion of 13q that was inherited from a maternal complex translocation between chromosomes 2, 13 and 20.

A 2 1/2 month old girl received genetic evaluation due to unusual clinical features and a small size for her age. A physical exam showed partial right side facial palsy, craniofacial dysmorphism (flat facies, flat occiput, small eyes, flat nose, long philtrum, a thin upper lip, small mouth), short neck, widely spaced nipples, a reduced chest circumference and long fingers and toes. The weight was 8lbs 10 oz (3%), OFC 36 cm (- 2SD), and the height 21 1/2 inches (5%). A cytogenetic study identified a del(13)(q12q14). FISH showed that the retinoblastoma gene was not deleted. The mother carried a complex rearrangement involving chromosomes 2, 13 and 20 which was clarified by FISH with chromosome-specific libraries. The segment 13q12.1-q12.3 was translocated to band 2p13. The segment 2p13-p25 was attached to the segment 13q12.3-q14.3. This latter segment was translocated to band 20p13. The father was not available for chromosome studies.

The phenotype of this child is consistent with other proximal deletions of 13q not extending into band q32 (lack of major malformations, minor anomalies, growth retardation and retinoblastoma if the RB locus is deleted). The prognosis is more favourable and the phenotype is mild compared to the manifestations of deletions involving band q32 (major malformations and severe MR and growth retardation) or the most distal deletions q33-q34 (severe MR without gross malformations or growth retardation).

Ring chromosome 17 with monosomy 17 associated with unusual severe malformations. *C. Baldermann¹, C. Taege², A. Musil², F. Rath², I. Hansmann¹.* 1) Institut fuer Humangenetik und Medizinische Biologie, Halle(Saale), Germany; 2) Institut fuer Pathologie; Halle(Saale) Germany.

We report on a premature female infant born at week 30 by caesarian section because of fetal bradycardia. The infant died 5 hrs pp. At autopsy severe craniofacial malformations were observed with microcephaly, hypertelorism, bilateral cheilognathopalatoschisis and dysmorphic ears as well as leftsided diaphragmatic hernia, cardiopulmonal malformation with agenesis of the lower lobe of the left lung, 2 ventricular septal defects and hypoplasia of the truncus pulmonalis. Genital anomaly with vagina and uterus duplex was present. Placental weight was too high and basal plate was too large in relation to gestational age. Maturation of villi was heterogeneous with areas of normal and large oedematous villi. Chromosome analysis of G-banded lymphocyte metaphases revealed a ring(17) in all 61 cells. In fibroblasts the ring(17) was confirmed in 36/40 cells. The remaining 4 cells (10%) disclosed monosomy 17 (45,XX,-17). FISH with probes specific for the short and long arm including subtelomeric regions was done. Signals were observed for each probe indicating that fusion of chromosome 17 arms was distal to the probe loci. Probes included that for Smith-Magenis (17p11.2) and Miller Dieker region (17p13.3), as well as for D17q25 (YAC659g5). To our knowledge there are only 10 documented cases of ring(17), 4 of them show features of Miller-Dieker syndrome and at least 2 presented with flecked retina suggesting that there may be a causal relationship between abnormalities of chromosome 17 and retinal pigment dysfunction. Several patients had mild nonlethal features in common such as growth, mental and motor retardation, microcephaly, mild facial dysmorphism, cafe au lait spots, seizures and hypotonia (ring chromosome 17 syndrome). The complex and lethal malformations of our new case has not been reported so far with ring(17). Our case has only minor clinical symptoms in common with those of the ring 17 chromosome syndrome. Explanations such as mosaicism for monosomy 17, altered gene function due to loss of telomeres and unmasking of a recessive mutation will be discussed.

A new approach in interpretation of comparative genomic hybridization (CGH) profile analysis: existence of two patterns of shifts of ratio profiles for specific chromosomes and chromosomal regions within normal variations.

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CGH technology has proven to be a powerful molecular cytogenetic diagnostic tool. Despite many thorough descriptions of CGH technique, the use of new approaches in the interpretation of profiles are needed to improve the efficacy and utility of CGH as a clinical diagnostic method. Our audit of 552 CGH analyses known to have normal diploid complements illustrates a tendency for the image profiles of certain chromosomes and chromosomal regions to display a particular pattern of deviation. The CGH was performed with the test DNA directly labeled with FITC (green) and reference DNA with TRITC (red). Our study shows that there are two distinct patterns of deviations involving certain chromosomal regions and whole chromosomes. Chromosomal regions 1p32-pter, 12q24-qter, 16p, and chromosomes 17,19,20,22 show a statistically significant tendency to exhibit a green-to-red ratio profile <1 , whereas chromosomal regions 3q, 4q, 5q, 6qcen-q24, 9p, 18q and chromosome 13 demonstrate a converse behaviour, green-to-red ratio >1 . When multiple chromosomes display simultaneous deviation within a CGH profile, all chromosomes of the same pattern deviate to the same side and all chromosomes of opposite patterns deviate to opposite sides. The behaviour of profiles of these chromosomes is not random, but predictable due to innate differences between G-band light and dark regions (A/T and C/G rich respectively). Our observations provide a new approach in evaluation of the green-to-red ratio profiles within the threshold levels for gains and losses (ratio of 0.8-1.2). Any discordance in behaviour of chromosomal regions of a certain pattern must be considered significant and indicative of abnormal chromosomal complement. Confirmation of the abnormal finding by means of other cytogenetic molecular techniques should follow.

Delineation of the dup 5q phenotype by molecular cytogenetic analysis in a patient with dup 5q/del 5p (Cri du chat). *B. Levy*¹, *T.M. Dunn*¹, *J. Kern*², *K. Hirschhorn*¹, *N.B. Kardon*¹. 1) Department of Human Genetics and Pediatrics, Mt Sinai Medical Ctr, New York, NY; 2) Department of Pediatrics, Flushing Hospital Medical Ctr, Queens, NY.

An infant girl presented with growth retardation, low set ears, prominent nose, short philtrum, microcephaly, low set nipples, significant VSD, bilateral clinodactyly of the 5th digits and a mewing cry. Cytogenetic studies of peripheral blood lymphocytes showed a 46,XX,add5p karyotype. Parental chromosome analysis revealed that the mother carries an apparently balanced pericentric inversion of chromosome 5, 46,XX,inv(5)(p15.2/3?q35.1?). In view of the patient's mewing cry, initial fluorescence in situ hybridization (FISH) studies using a commercial Cri du chat probe (D5S721, D5S23), which maps to 5p15.2, revealed signals on both the normal and derivative chromosomes. Further FISH analysis was performed to determine the nature of these rearrangements. Telomeric probes specific for 5p and 5q were used to confirm the pericentric inversion in the mother and demonstrated the loss of the terminal 5p region and a duplication of the terminal 5q region in the proband. Comparative genomic hybridization (CGH) was utilized to further refine both breakpoints in the recombinant chromosome 5, and clearly places them at 5p15.3 and 5q34. Therefore, the derivative chromosome found in the proband arose during a recombinant meiotic cross-over event in the mother leading to loss of material from 5p15.3@pter and a duplication of 5q34@qter. The presence of the cat-like cry appears to be the only specific feature that can be linked to the loss of 5p material. The remaining dysmorphic features in this infant appear to be due specifically to the duplication of the 5q sequences. The combination of FISH, CGH and cytogenetics has helped correlate the spectrum of clinical phenotypes found in cri du chat and 5q duplications with specific chromosome breakpoints. We have confirmed that the characteristic cry of the Cri du chat syndrome is due to the deletion of the most distal part of the classic del 5p. More importantly we have defined the duplication of 5q34@qter as a distinct clinical phenotype.

Supernumerary Ring Chromosome 11 Mosaicism causing retinal colobomas. *E.W. McPherson^{1,2}, M.M.*

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Ring chromosome 11, with clinical features similar to del 11q, has been reported in several patients, but we are aware of no previous descriptions of a supernumerary ring chromosome 11. We report an infant with a supernumerary ring 11 in 28% of her cells. Dandy-Walker variant was diagnosed prenatally, but the parents declined amniocentesis and the patient was born at term with a weight of 3081g, length of 48 cm and OFC of 32cm. She had an unusual appearance with marked hypertelorism, short downslanting palpebral fissures, down-turned mouth, thin lips, small nose and cupped ears. Eye exam showed bilateral retinal colobomas. She failed a hearing screen. Echocardiogram showed a small VSD which has been asymptomatic. MRI showed hypoplasia of the corpus callosum, increased fluid in the middle cranial fossa, migration abnormality and fetal prosencephalic vein. Despite gastroesophageal reflux, her postnatal growth has been normal. She was hypotonic as a neonate, but her development at 2 months appeared normal except for inconsistent visual fixation. Her karyotype was 47,XX,+ring11/46,XX. Parental chromosomes were normal. The family history was remarkable only for a stillborn sibling with no obvious malformations, who was not karyotyped. The supernumerary ring in our patient involves the middle 2/3 of the chromosome. She has mosaic trisomy for proximal 11p and proximal 11q and her clinical features overlap those of a previously reported patient with dup 11p12-13 who had minor dysmorphic features, retinal colobomas and mild developmental delay. The eye abnormalities may be attributable to duplication of the PAX6 gene. There is also overlap with a previously reported patient who had dup 11q13.3-14.2 with mild to moderate retardation and congenital heart disease. The larger duplication in our patient presumably explains her more significant dysmorphism. Complete trisomy 11 has never been reported in any liveborn individual. Our patient's mosaicism has allowed her to survive with a duplication of chromosome 11 larger than any previously reported.

There is High malignancy risk in Mosaic Variegated Aneuploidy Syndrome. *S. Jacquemont¹, M. Boceno², D. Genevieve⁴, J.M. Rival², F. Mechinau³, A. David¹.* 1) Unite de genetique clinique, Service de genetique medicale Hpital mere enfant, Nantes, France; 2) Unite de cytogenetique antenatal, Service de genetique medicale, Centre Hospitalier Universitaire de Nantes, France; 3) Service d'oncologie pediatrique, Hopital mere enfant, Nantes, France; 4) Service de genetique medicale, Hopital Necker-Enfants Malades, France.

14 cases of mosaic variegated aneuploidy (MVA) have been reported in the last 10 years. The phenotype of this rare syndrome has been quiet consistent : sever microcephaly, growth deficiency, mild dysmorphogenesis and mental retardation. We describe here a young boy who associates those clinical features, constitutional MVA and myelodysplasia with a monosomy 7 bone marrow clone. At the age of three, his myelodysplasia has transformed into an acute lymphoblastic leukemia and he died soon after. Hematological findings have never been described in the MVA syndrome. Patients described by Kadjii et al [1998], Limwongse et al [1999] and us are the only 3 out of 14 cases (21%) with neoplastic findings. Nevertheless it is easily understandable that somatic cells suffering mitotic instability can at one point become malignant by increased expression of oncogenes or decreased expression (haploinsufficiency) of tumor suppressor gene. Indeed, multiple chromosome aneuploidy are commonly found in solid tumor and in hematological malignancies. More over, many reports mention very young patients (65% of the patients described are under 4 years old) and there long term-follow up might reveal malignancy. It is clear now that patients with MVA have a high malignancy risk. Therefore, if other cases of cancers and hemopathies are reported, MVA could become a neoplastic predisposing syndrome.

Fragile site 16(q22): is there a phenotypic consequence? *A. Hing, K. Leppig.* Div Genetics & Dev, Univ Washington, Seattle, WA.

The fragile site at 16q22 [fra(16)(q22)] is rare fragile site, sensitive to distomycin, results from an amplification of an AT-rich minisatellite repeat and is thought to be without phenotypic sequelae. We report a four month old female with microstomia, bilateral cleft lip, cleft palate, micrognathia, broad thumbs and clinodactyly. Routine cytogenetic analysis performed on peripheral blood lymphocytes showed expression of the fragile site at 16q22 in 41 of 101 metaphase cells examined, with six of the metaphase cells showing a deletion of 16q22 to qter. Family history is positive for a paternal aunt and cousin with oral clefting.

Oral clefting has been characteristic of other patients with fra(16)(q22) and an abnormal phenotype. A family reported by Bettex (et al. *Eur J Pediatr Surg* 8:4-8, 1998) describes a three generation family with cleft palate, microstomia, hypoplasia of the mandible and partial anodontia, segregating with fra(16)(q22). Another family reported by Dummer (et al., *Am J Med Genet* 16:277-284, 1983) described two sibs with fra(16)(q22), one with cleft palate and brachydactyly, and the other with three cranial fontanelles, congenital heart disease, and syndactyly of toes 2-3. Their healthy mother also had fra(16)(q22). Patients with isolated deletions involving 16q22.1-q24.1 (Callen et al., *J Med Genet* 30:828-832, 1993) have anomalies that include oral clefting. Prescott (et al., *Hum Genet* 106:345-350, 2000) has identified 16q22-24 as one of nine areas of interest by non-parametric linkage scores for nonsyndromic cleft lip with or without cleft palate.

The presentation of this patient and review of literature suggest that susceptibility genes for orofacial clefting may reside on chromosome 16q22 and a possible causal relationship between fra(16)(q22) and the development of orofacial clefts.

Tricuspid atresia in mosaic tetrasomy 8p. *P.R. Koduru, R. Schiff, A. Yenamandra, E. Arnold, M.G. Bialer.* Dept Pathology and Pediatrics, North Shore Univ Hosp/NYU School of Medicine, Manhasset, NY.

A 5 mo old girl from the Dominican Republic was seen for evaluation and treatment of congenital heart disease (CHD). She had presented with breathing difficulties at 1 day of age and a chest X-ray at 1 wk revealed vertebral anomalies. She had poor weight gain and tired during feeding. Admission weight was 3.64 kg (<<5%), length 60 cm (5%) and OFC 38.8 cm (5%). Dysmorphology exam was significant for large anterior fontanelle, unfolded right ear with soft cartilage, mild 5th finger clinodactyly, hypoplastic proximal phalanx of left 5th finger, and 2nd toe overriding 3rd bilaterally. Chest X-ray revealed butterfly and hemivertebrae involving T6-10. Ribs were somewhat irregular. Echocardiography demonstrated tricuspid atresia, a non-restrictive atrial defect, and a very restrictive bulboventricular foramen (VSD). Oxygen saturation was in 50's-60's. Head ultrasound showed agenesis of the corpus callosum (ACC). Chromosome analysis revealed 12/62 cells with a large metacentric marker chromosome. Banding pattern and fluorescence *in situ* hybridization with a chromosome 8 specific painting probe WCP8 (Vysis Inc.) proved the marker to be derived from 2 copies of the short arm of chromosome 8 (mos 47,XX,+mar[12]/46,XX[50].ish i(8)(p10)(WCP8+)). She underwent atrial septectomy and bidirectional right superior vena caval to pulmonary artery anastomosis. Her post-operative course was complicated by RSV bronchiolitis, thrombosis of the left innominate vein causing multiple pulmonary emboli, and several episodes of bacterial sepsis. She died at almost 7 mo. Mosaic tetrasomy 8p has been described in 11 previous cases in the world literature. Including this case, 6/12 (50%) have had CHD. Tricuspid atresia has not been previously reported in this condition, but mitral atresia has been seen once. Vertebral segmentation defects have been seen in 9/11 (81%) and ACC in 7/9 (78%) individuals studied. Mild-moderate mental retardation has been reported in 6/7 surviving individuals (Am J Med Genet 1997;73:330). Additional cases will be helpful in delineating the features of this syndrome.

Inherited chromosome Xp22.3 deletion: Discordant phenotype in mother and daughter. *B. Mouron^{1,3}, M.L.*

Begleiter^{1,3}, R. Rinaldi^{2,3}, M.G. Butler^{1,3}, J.H.M. Knoll^{1,3}. 1) Section of Medical Genetics and Molecular Medicine; 2) Section of Pediatric Rehabilitation Medicine; 3) Children's Mercy Hospital and the University of Missouri Kansas City School of Medicine, 64108.

Terminal deletions of the X chromosome short arm are often associated with short stature and occasionally with mental retardation. We present the chromosomal findings on a 34 month old female who was referred because of small size (<5th %ile for height), facial dysmorphism and developmental delay, and on her mother with short stature (height 154cm, 5th%ile) and normal intelligence. Routine cytogenetic analysis of peripheral lymphocytes from both individuals showed similar deletions of one X chromosome at Xp22.3. FISH analysis with chromosome Xp22.3 specific probes for the Kallman gene (KAL1), steroid sulfatase gene (STS) and the Xp subtelomeric region demonstrated 46,X,del(X)(p22.3). ish del(X)(p22.3)(DXZ1+,KAL1+,STS-,TELXp-). The KAL1 locus was intact, but STS and the subtelomeric sequence were deleted. Such a deletion in males would result in an abnormal phenotype including ichthyosis, whereas, in females, a normal phenotype would be likely. The patient's father was normal and therefore was unlikely to have transmitted a recessive X-linked gene unmasked by the deletion. In deletion positive females with clinical abnormality, the abnormality may be due to preferential X chromosome inactivation of genes or a reduction in gene expression for those genes that escape inactivation (ie. STS and KAL1). We are evaluating possible skewed X-inactivation by replication banding and allele- specific replication timing.

Prenatal diagnosis and characterization of an unbalanced whole arm translocation, der(18;22)(q10;q10), resulting in monosomy for 18p. *E.M. McGhee¹, Y. Qu², P.D. Cotter^{1,3}*. 1) Dept Pediatrics, Medical Genetics, University of California, San Francisco, San Francisco, CA; 2) Chapman Institute of Medical Genetics, 5300 East Skelly Drive, Tulsa, OK; 3) Division of Medical Genetics, Children's Hospital Oakland, 747 Fifty Second Street, Oakland, CA.

Monosomy for the short arm of chromosome 18 is one of the most frequent autosomal deletions observed. As many as 16% of cases were reported to be due to an unbalanced whole arm translocation resulting in monosomy 18p. The origin and structure of these derivative chromosomes was reported in only a few cases. We report the prenatal diagnosis and characterization of a new case of monosomy 18p due to an unbalanced whole arm translocation. Amniocentesis was performed at 16 weeks of gestation on a 34 year old woman who was initially referred for advanced maternal age and holoprosencephaly identified by ultrasound. Karyotype analysis showed an unbalanced whole arm translocation between the long arm of one chromosome 18 and the long arm of one chromosome 22, 45,XX,der(18;22)(q10;q10), in all metaphases. In effect, the fetus had monosomy for 18p. Parental karyotypes were normal, indicating a de novo origin for the der(18;22). FISH analysis was performed with alpha satellite probes D18Z1 and D14Z1/D22Z1 to identify the origin of the centromere on the der(18;22). Signal was observed with both probes, indicating that the der(18;22) was a dicentric chromosome. Genotyping of the fetus and her parents with chromosome 18p STS marker D18S391 showed only the paternal 187 bp allele was present in the fetus, indicating the chromosome 18 involved in the der(18;22) was maternally derived. While familial whole arm translocations were more likely to be monocentric, this case and previous reports demonstrate that de novo unbalanced whole arm translocations are most often dicentric rearrangements. Loss of the reciprocal translocation product, resulting in monosomy for 18p, is likely a result of instability due to little or no centromeric material on that derivative chromosome.

Complex counseling issues associated with a balanced, nonreciprocal 12;15 translocation. *K.A. Kaiser-Rogers¹, K.K. Phillips², C.M. Powell¹, M.I. Roche¹, K.W. Rao^{1,2}.* 1) Dept Pediatrics, Univ North Carolina, Chapel Hill, NC; 2) Dept Pathology, Univ North Carolina, Chapel Hill, NC.

An unusual, balanced, nonreciprocal 12;15 translocation was detected in a phenotypically normal 30 year old woman ascertained because of multiple pregnancy losses. This patient's balanced karyotype [45,XX,der(12)t(12;15)(q24.33;q11.2)] contains 45 rather than 46 chromosomes and the entire long arm of one chromosome 15 homologue appears to have been translocated to the distal long arm of chromosome 12. A chromosome 15 a-satellite FISH probe did not hybridize to the der(12) chromosome, while the most proximal Prader-Willi (PWS)/Angelman syndrome (AS) probes did, suggesting that this chromosome is monocentric with the chromosome 15 breakpoint lying proximal to the PWS/AS region. Subtelomeric and pan telomeric probes hybridized to the derivative chromosome showing that little, if any, DNA was lost from the distal long arm of chromosome 12. No evidence of mosaicism was seen in the 100 lymphocytes that were examined.

The genetic counseling issues involving this translocation are complex. This patient is at increased risk for conceiving pregnancies with unbalanced karyotypes secondary to chromosome malsegregation. In this case, monosomy or trisomy for chromosome 12 or 15 could occur; however, these are lethal abnormalities and her risk for having live-born children with these karyotypes is low. Since malsegregation followed by correction can result in an abnormal phenotype secondary to uniparental disomy (UPD), this issue was also discussed. Trisomy correction could produce a fetus with maternal UPD for chromosome 12 or 15. Although no UPD 12 cases have been reported, maternal UPD 15 causes PWS. Similarly, monosomy correction would result in paternal uniparental isodisomy (UPiD) 15 and AS. The risk of inheriting a recessive disorder secondary to UPiD was also considered. Finally, the tendency for familial translocations involving the PWS/AS region to produce deletions involving proximal chromosome 15 was discussed. These deletions appear to arise by unequal crossing over; the consequence of such a deletion in this patient's offspring would be AS.

Subtelomeric testing for cryptic chromosomal rearrangements in 68 patients with idiopathic mental retardation and dysmorphology. *X.L. Huang¹, H.E. Wyandt^{1,2}, J.M. Milunsky^{1,3}*. 1) Human Genetics, Boston Univ Sch Medicine, Boston, MA; 2) Pathology, Boston Univ Sch Medicine, Boston, MA; 3) Pediatrics, Boston Univ Sch Medicine, Boston, MA.

Sixty-eight patients have been referred to our center for subtelomeric testing. In the course of these studies we used either the subtelomeric Cytocell 41-probe device (Rainbow Scientific, Windsor, CT) or a panel of 41 individual TelVision probes (Vysis, Downers Grove, IL). Individual probes from either source were used when some probes had to be repeated. The majority of cases were first studied by high-resolution chromosome banding. In some cases, other tests were also done to rule out possible causes of mental retardation (mr) and/or dysmorphic features. Of 23 patients examined by one of us (JMM), 12 have moderate to severe mr and 11 have mild mr that eluded explanation by other testing. From this group, three (13%) have *de novo* deletions of subtelomeric sequences. Case 1, a 10.5 y.o. male with a terminal deletion in 1p36 has severe mr with lack of language, self-abusive behavior, hypotonia as an infant, esotropia, growth delay, cerebral atrophy and dysmorphism. Case 2, a 2 y.o. male with a subterminal deletion in 9q34 has severe mr, central apnea, hypotonia, optic nerve atrophy, multiple dysmorphic features, multiple cardiac defects and bilateral hearing loss. Case 3, an 18 y.o. male with deletion in 10q26, has moderate mr, autistic behavior, bipolar disorder with multiple suicide attempts and dysmorphism. Of 45 cases sent from other clinics, one (Case 4), an 18 mo female with severe mr, hypotonia, gastroesophageal reflux and failure to thrive, has deletion in 2q37. The clinical details of all four cases will be presented. Case 1 represents a well-described subtelomeric deletion. Cases 2, 3 and 4 represent less frequently described deletions. The finding of these four cases is consistent with previously reported series and confirms that subtelomeric FISH testing is appropriate, especially for those cases with unexplained moderate to severe mr.

A case of complete tetrasomy 12p versus mosaic tetrasomy 12p (Pallister-Killian syndrome). *M.C. Lowery^{1,2}, R.L. Young², A. Li¹, M. Fowler¹, L.A. Prouty², M. Lucas³, H. Chen².* 1) Dept. Pathology; 2) Dept. Pediatrics; 3) Dept. Obstetrics & Gynecology, Louisiana State Univ Health Sciences Center, Shreveport, LA.

Pallister-Killian syndrome, or mosaic tetrasomy 12p, is a relatively rare syndrome that is clinically manifested by profound mental deficiency and multiple congenital anomalies. The syndrome exhibits tissue-specific mosaicism where a supernumerary isochromosome 12p is marked in fibroblasts but virtually absent in peripheral blood. Very few cases, if any, show total tetrasomy 12p. We report an isolated clinical case of a 18 year-old prima gravida who presented to the hospital in labor. Prenatal ultrasound revealed polyhydramnios, diaphragmatic hernia, short limbs, and short neck. Although the possibility of Pallister-Killian was suspected, amniocentesis was not performed because of late gestation and the patient being in labor. At birth, the infant was found to have hypertelorism, short neck, short limbs, and diaphragmatic hernia. In spite of resuscitation, the infant expired and an autopsy was performed. Death was attributed to pulmonary hypoplasia secondary to diaphragmatic hernia. Cytogenetic analysis on tissue from the infant revealed all cells with i(12p). Fluorescence in situ hybridization (FISH) was performed using a pericentromeric probe (CEP12/SpectrumOrange, Vysis) and a whole chromosome paint probe (WCP/SpectrumOrange, Vysis) for chromosome 12. FISH confirmed 4 copies of the chromosome 12 centromeric region in all interphase and metaphase cells analyzed. WCP analysis confirmed that structurally the der(12) was comprised of all 12 material. This case documents complete tetrasomy of 12p and that survival to term is possible.

Deletion of 2q37 and duplication of 10q24: Report of two cases in the same family and literature review. A.

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Two patients (first cousins, once removed) with dysmorphic features and developmental delay have an unbalanced translocation resulting in loss of 2q37.3-qter and duplication 10q24.1-qter. The patients were ascertained independently and it was initially unknown that they were related to each other. Cytogenetic and fluorescent in situ hybridization (FISH) analysis identified a der(2)t(2;10)(q37.3;q24.1) karyotype. Patient 1 is an 18 year old severely mentally impaired male with dolicocephaly, high arched palate, flat nasal bridge, acquired total alopecia, prominent ears, joint contractures and a prominent scapula. The family was lost to follow-up for 5 years after the initial visit. After the association with patient 2 was identified, the family was able to be recontacted. The mother is an obligate carrier and the only sibling had a 46,XX karyotype. Patient 2 is a 12 month old female with craniosynostosis, flat nasal bridge, low set and posteriorly rotated ears with delayed development of approximately 2 months. A positive family history was initially denied by the family. After the karyotype results became known, the family history was provided. The father's karyotype is 46,XY,t(2;10). Previously described features of patients with deletions of chromosome 2q37 include moderate mental retardation, depressed nasal bridge and hand and feet abnormalities. Duplication 10q is characterized by mental and growth retardation and dysmorphic features, including short palpebral fissures, hypertelorism, malformed ears and broad nasal bridge. This is the first report of patients with the both loss of 2q and duplication of 10q. Both patients described have unusual phenotypic features not previously reported in patients with a 2q deletion or 10q duplication. These patients emphasize the importance of understanding genetic information and risks, and sharing it among their extended family.

Ring X chromosome and male infertility - Report of a case 45,Y/46,Y,r(X). *G. Quenum¹, M. Freund¹, M.*

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Ring X chromosomes are usually small, present as a mosaic in patients with Turner syndrome. In male patients, large ring X has been reported only once associated to short stature in a 15 years old boy. We report on a second patient, the first known for his infertility, with severe oligozoospermia and short stature. The karyotype showed a large X ring with apparently no deletion on G banding. The ring was also present on fibroblast culture. Using whole chromosome painting and subtelomeric probes we demonstrated by FISH that the subtelomeric regions were preserved. To our knowledge, telomere-to-telomere fusion has not been described for X ring chromosomes. Different lymphocyte culture times (48 h, 72 h, 96 h) showed that the ring was unstable and lost in 7% of cells in cultures of more than 48 hours with a karyotype 45,Y. Short stature could be related to the instability of the ring. The ring seems to have been inherited from his mother whose karyotype revealed 73 cells 45,X, 26 cells 46,XX and a unique 46,X,r(X) cell. In spite of her karyotype she had four pregnancies without any problem. The status of fertility of patients with ring chromosome is not well known, however, azoospermia has been previously described as the only feature in some reports of male adults with a ring 21. This is the first report of a male patient whose infertility could be related to the presence of a ring X chromosome, and the first demonstration by FISH analysis of the preservation of subtelomeric sequences for a ring X chromosome.

Assessment of subtelomeric regions of children with autism: Detection of a 2q deletion. *D.J. Wolff, K. Clifton, J. Charles.* Departments of Pathology/Laboratory Medicine, Medical Laboratory Sciences and Pediatrics, Medical University of South Carolina, Charleston, SC.

Autism is a behaviorally defined syndrome characterized by difficulties in socializing, communicating, and by the presence of stereotypical behaviors. The disorder is a complex genetic trait with no known predisposing genes. We have utilized a multiprobe system that includes probes for the subtelomeric regions of all of the chromosomes to screen for cryptic cytogenetic abnormalities in ten children with a diagnosis of autism and a previous normal standard cytogenetic analysis. An apparent deletion of 2q was detected in one patient. A targeted high resolution study revealed a subtle abnormality on one chromosome 2 homologue. This patient's mother's chromosomal analysis and subtelomeric assessment were normal and the father was unavailable. There have been several reports of children with dysmorphic features, autistic behaviors and deletions of 2q detectable by standard techniques. It may be that the distal region of 2q harbors a gene or genes important for normal brain function. Further studies are underway to define the extent of the deletion in this patient. Also, the pilot project is being extended to include additional autistic children to determine if deletions of 2q are a consistent finding and/or to potentially discover new regions that may include genes important in autism. The results of our study suggest that subtelomeric deletions may be involved in the pathogenesis of a significant percentage of autism cases (10% of our unselected pilot population).

Complex chromosomal rearrangements in a 1p36 deletion syndrome child with a cryptic interstitial deletion. C.-H. Tsai¹, J. Yu², T. Gilfillan², L. Meltesen², B. Hirsch³, L. McGavran². 1) Division of Genetics Services, The Childrens Hospital, Denver, University of Colorado Health Sciences Center, CO; 2) Colorado Genetics Laboratory, Departments of Pathology & Pediatrics, UCHSC & TCH; 3) University of Minnesota Medical School.

We report a girl with a distal 1p36 deletion phenotype and a complex rearrangement of 1p resulting in a cryptic deletion. Born at term and appropriately grown, she was hypotonic and had torticollis, and left iris and inferior retinal coloboma. In utero, mildly decreased fetal movement had been noted. Screening demonstrated hearing loss. Brain MRI showed right plagiocephaly and mildly delayed myelination. At 18 months physical examination revealed prominent forehead, mid-face hypoplasia, flat nasal bridge, deep-set eyes with hypertelorism, high-arched palate and bilaterally short fifth fingers. Growth parameters were between 25th to 50th percentile, and she was hypotonic with decreased muscle bulk. Other findings included hypothyroidism, thrombocytosis, multiple ear and respiratory infections, and global developmental delay. High resolution cytogenetic studies revealed an abnormality of 1p34->pter. Parental cytogenetic studies were normal. FISH studies showed an unexpected reciprocal translocation of subtelomeric sequences between 1p and 16p, and loss of D1Z2 sequences on the der(1). We constructed a mini-paint microdissection probe for bands 1p36.1-1p36.3. It hybridized more proximally on the der(1p) and gave a smaller signal, consistent with an inversion and deletion of part of these bands. The karyotype is 46,XX,der(1)del(1)(p36.2p36.3)inv(1)(p36.3p36.1),t(1;16)(pter;pter). Heteromorphisms of 1qh show the der(1) to be of paternal origin. As a result of the initial cytogenetic findings, a diagnosis of 1p36 deletion syndrome was confirmed by closely comparing the phenotypes of reported 1p36 deletion cases and extensive molecular cytogenetic studies to prove a deletion in this complex rearrangement. This case illustrates the importance of recognizing characteristic physical features and aggressive laboratory confirmation to provide accurate syndromic diagnosis.

Euchromatic 16p+ heteromorphism: a rare variant or chromosomal abnormality:. *T. Shklovsky, S.M. Kleyman, M.J. Macera, R.S. Verma.* Department of Molecular Medicine & Genetics, Wyckoff Heights Medical Center, Brooklyn New York Hospital/Weill Medical College of Cornell University, New York, N.Y.

Chromosomes 1, 9 and 16 display the highest morphological variation due to the presence of a heterochromatic block in the secondary constriction region [qh]. Numerous types of variations of chromosome 9 have been observed. The qh region of chromosome 1 also displays an additional G-negative band that is inherited by progeny. The rarest variant is chromosome 16 where the short arm has additional G-negative material and thus presents as a metacentric chromosome. There are a number of reports that suggest that these morphological variations have no clinical significance because minor malformations observed in these patients are not observed in normal family members with the same variant chromosome. Recently, we were referred a newborn male with a high-arched cleft palate and micrognathia. A clinical diagnosis of Pierre-Robin syndrome was suspected. Cytogenetic findings by GTG-banding revealed: 46,XY[135]/46,XY,16p+[5]. i.e. an enlarged short arm of chromosome 16 was noted in 5 cells. FISH with a whole chromosome painting probe for 16 (Vysis) gave a positive signal over the entire der(16), confirming that the additional material was derived from chromosome 16. The mosaic cell line was most likely caused by post-zygotic mutation. It has been hypothesized that its origin is similar to that of facultative heterochromatin of the inactive X-chromosome and may have differential regulation of gene activity [Monk, 1987; Thompson et al, 1990]. At present, the parents are not available for cytogenetic evaluation. This heteromorphism was first noted by Thompson and Robert (1987) and confirmed by many investigators, although, not in a mosaic state. The possibility exists that this mosaic may be an artifact due to cultural conditions. This case is the first one that presented with severe malformations. Genetic counseling of de novo cases is very difficult especially if they are ascertained via genetic amniocentesis.

A range of phenotypes in three patients with a karyotype of 45,X/46,X,idic(Y)(q11.2). *A.L. Zaslav¹, J. Jacob¹, S. Allan², D. Blumenthal², J.E. Fox², P. Papenhausen³*. 1) Dept Pathology, Long Island Jewish Medical Ctr, New Hyde Park, NY; 2) Dept of Pathology, Long Island Jewish Medical Center, The Long Island Campus of the Albert Einstein College of Medicine, New Hyde Park, NY; 3) Laboratory Corporation of America, Research Triangle, North Carolina.

Patients with a 45,X cell line and an idic(Y) are uncommon. Reported phenotypes include: Turner syndrome (TS), virilized female, normal male, and males with ambiguous genitalia (AG). We report 3 cases demonstrating this variability. Case 1: a 33 y female had amniocentesis for AG at 32 wk US. The karyotype was 45,X[28]/46,X,?Y[1]. Interphase FISH analysis revealed an idic(Y)(q11.2) in 9 of 315 nuclei. Postnatal studies revealed a 38 wk baby with AG and features of TS. Postnatal cytogenetics on blood revealed 45,X[48]/46,X,idic(Y)(q11.2)[17]. Interphase FISH analysis revealed 45,X/46,X,idic(Y) in the following ratios: blood (55/10), tissue (110/6), and left gonad (50/3). Molecular analysis using 4 long arm spermatogenesis Y probes demonstrated the presence of DYS275, SYS209 and deletion of DAZ and SPGY. The dual presence by FISH of centromeres and SRY loci confirmed the idic(Y). Case 2: a 37 y female had amniocentesis for AMA. Cytogenetics and FISH revealed a de novo 46,X,idic(Y)(q11.2)[15]/45,X[10].ish idic(Y)(q11.2)(SOCEPY++). Molecular analysis using the same Y probes as in Case 1 demonstrated the absence of DYS209, DAZ, and SPGY. Two centromeres and SRY loci found by FISH confirmed the idic(Y). High-resolution US and postnatal clinical evaluation revealed a normal appearing male. Case 3: a 17 y female was referred for delayed puberty and short stature. A karyotype of 46,X,idic(Y)(q11.2)/45,X was seen in blood (94/6), skin (48/52), and left ovary (2/48). The low percentage of Y bearing gonadal cells justified the phenotype. These 3 cases demonstrate the wide range of variability among patients with this karyotype. Sexual development in patients with this abnormality depends on the gonadal cell line distribution and the genes deleted on the idic(Y). With current technology, correlation of phenotypic, cytogenetic, and molecular data will be useful in predicting fertility and phenotypes.

The 22q11.2 Deletion: Parental Origin and Meiotic Mechanism. *S.C. Saitta, D.A. Driscoll, E.F. Rappaport, D.M. McGinn, E.H. Zackai, B.S. Emanuel.* Dept Human Genetics, Childrens Hosp Philadelphia.

Microdeletions of 22q11.2 are associated with velocardiofacial/DiGeorge syndrome (VCFS/DGS). It is the most frequently occurring deletion syndrome in humans with an estimated incidence of 1:4000 live births. The molecular mechanism by which the deletion occurs is largely unknown. Analysis of our patients by fluorescence in situ hybridization (FISH) demonstrates a standard deletion of ~3 Mb in 80-90% of patients. The overwhelming majority of deletions occur as de novo lesions indicating an extremely high mutation rate within this genomic region. The 22q11 deletion interval contains at least four large low copy repeats (22-LCRs) which coincide with the recurrent deletion endpoints (DEPs), strongly implicating them in the events leading to deletion. In order to further investigate the meiotic mechanism of deletion formation, we have examined families in which de novo standard 22q11.2 deletions occurred. We used 5 microsatellite markers within the deletion to determine the parental origin of the deleted allele in 59 cases. Thirty were of paternal origin, while 29 were of maternal origin, demonstrating no bias. For further analysis we designed PCR primers for polymorphic markers F8VWFP and D22S420, which flank the proximal standard DEP, and markers D22S257 and TOP1P2, flanking the distal DEP to amplify genomic DNA from ten 3-generation families. Segregation analysis using grandparental haplotypes and one proximal and one distal marker, was consistent with a meiotic interchromosomal cross-over event in at least two informative families. This is in agreement with ten previously published cases which indicated such events as leading to the deletion. Our study is the first in which the size of the deletion has been determined as part of the analysis. We are currently analyzing the remaining families and collecting additional ones in an effort to understand the meiotic recombination events which lead to the deletion.

Prenatal detection of an alphoid marker chromosome; recommended adjunctive testing. *P.R. Papenhausen¹, R. Wiltshire², J.H. Tepperberg¹, P.N. Mowrey¹, I.K. Gadi¹, K.K. Phillips¹*. 1) Dept Cytogenetics, Labcorp of America, Res Triangle Pk, NC; 2) Dept Pathology, Duke U Med Cnt, Durham, NC.

Reports of marker chromosomes without standard alphoid centromere associated repeats have escalated due to the increasing availability of FISH techniques to quickly resolve chromosomal origin. A 37 yo G2PO woman referred for amniocentesis for AMA revealed a non-mosaic 47,XX,+mar karyotype. Level II US and an echocardiogram were normal. The de-novo marker was C-band negative, alphoid in systematic analysis (Cytocell), and appeared to be a small ring (1/4 size of chr. 21). Origin was determined by systematic paint probe analysis (Cytocell), revealing chromosome 10 positivity consistent with complete unique sequence (euchromatin) content. Previous reports (40) of alphoid markers with neocentromeres (neos) have included three cases derived from chromosome 10. The locations of neos appear to cluster at specific sites on select chromosomes #13(q32), #15(q25), #3(q27). The frequent involvement of telomeric regions suggests that the use of subtelomere probes will be helpful to further characterize such markers, followed by regional cosmid probes. Localization efforts will include CGH, which could benefit from the frequent inverted duplication structure of alphoid markers, yielding partial tetrasomy levels. We will also perform microdissection/reverse FISH to the patient's metaphase chromosomes. If both chr 10 homologues (and marker) are positive, this will offer exclusion of a balanced deletion/marker generation that has been reported in 7 cases. No deletion of a chr.10 homologue could be visualized in the present case, so the risk of anomalies/MR due to partial trisomy or tetrasomy is substantial. The present array of ancillary tests allows increasingly valuable tools for prognosis. Standardizing such testing to allow reasonable clinical comparisons will be necessary before defined risks can be generated.

Molecular Analysis of Syndromic Congenital Heart Disease by Using Short Tandem-Repeat Polymorphic

Markers. *Y.R. Shi¹, K.S. Hsieh⁵, J.Y. Wu^{1,3}, C.C. Lee^{1,4}, C.H. Tsai^{1,2}, F.J. Tsai^{1,2,3}*. 1) Department of Medical research, china medical college hospital, Taichung, Taiwan; 2) Department of Pediatrics, Veterans General Hospital, Haohsiung, Taiwan; 3) Medical Genetics department, China Medical College Hospital, Taichung, Taiwan; 4) Neurology department, China Medical College Hospital, Taichung, Taiwan; 5) Pediatrics department, China Medical College Hospital, Taichung, Taiwan.

Velocardiofacial syndrome (VCFS), DiGeorge syndrome (DGS) and CATCH22 are developmental disorders characterized by conotruncal heart defects. Most of them have hemizygous deletions within chromosome 22q11, suggesting that haploinsufficiency in this region are responsible for their etiologies. To effectively understand the molecular basis for the chromosomal deletions, we designed a semi-quantitative fluorescent PCR using 11 highly polymorphic markers located in 22q11 to do genotyping analysis on ten probands (five VCFS, two DGS and three CATCH22) and their unaffected relatives. We found two VCFS, two DGS and two CATCH22 patients have a 3-Mb deletion. One patient with CATCH22 had a 1.5-Mb deletion and a crossover occurred in the same interval at the other allele. These results suggested that the specific regions in 22q11 are susceptible to rearrangement and the deletions might be the genetic etiology of these three syndromes. It also revealed that the deletion size is not correlated with the severity of the clinical manifestations.

Identification of extra material on the long arm of chromosome 5(q33-qter) by FISH technique. *V. Jobanputra¹, M.J. Macera², A. Kriplani¹, R.S. Verma², K. Kucheria¹.* 1) Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India; 2) Division of Molecular Medicine and Genetics, Wyckoff Heights Medical Center, Brooklyn-New York Hospital/Weill Medical College of Cornell Medical Center, New York, NY.

A 28 year-old, gravida 4, para 3 with poor obstetric history presented with severe oligohydramnios with bilateral polycystic kidney disease in the fetus. Cytogenetic analysis performed from the cord blood sample of the fetus showed the presence of additional material on the long arm of chromosome 5 in 25% of the metaphases analyzed. This additional material was suspected to be the bands 5q33-qter. Nevertheless, the initial impression cannot be related to a specific clinical presentation as variable features are associated with these bands. In an attempt to verify this duplication, the FISH technique, using a whole chromosome painting probe specific for chromosome 5 (wcp5, Vysis), was applied to metaphases from fetal tissue. Hybridization signals were detected over the entire derivative chromosome 5, confirming that the additional material was indeed 5q33-qter. The karyotype was rewritten as 46,XY/46,XY,dup(5)(q33q35).ish(wcp5+). Since the duplicated segment is very small, the clinical findings observed in this case may be associated with these chromosomal bands. Although patients with dup5(q11-15) have been reported to have renal and urinary tract abnormalities, these abnormalities have not been previously reported in association with dup5(q33-qter). A review of other cases with variable duplicated segments has been tabulated in an effort to establish a genotype/phenotype relationship.

Cryptic subtelomeric translocation t(2;16)(q37;q24) segregating in a family with unexplained stillbirths. D.

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We report on a familial submicroscopic translocation affecting the subtelomeric regions of chromosomes 2q and 16q. The index case, a six month-old male, presented with mild craniofacial dysmorphisms and psychomotor retardation. A conventional karyotype, performed at birth, did not reveal chromosome abnormalities. As family history showed along three-generations stillbirths and the presence in a few liveborns of mild non specific dysmorphic features, suggesting segregation of a familial translocation, a FISH study with chromosome-specific subtelomeric probes was performed. FISH analyses identified a familial cryptic translocation t(2q;16q), with an unbalanced form in the proband and a balanced form in the mother and the maternal grandfather. The unbalanced rearrangement leads to trisomy of the telomeric region of chromosome 16q and the concurrent monosomy of the distal region of chromosome 2q. An unbalanced form of the translocation is suggested by the family tree to underlie the observed stillbirths. The unbalanced translocation seen in the proband is inferred to have been present in a maternal aunt who also had the craniofacial dysmorphisms of the index case and deceased at 14 months of age. The grandfather, a balanced translocation carrier, is affected by late onset macular degeneration (MD) and, as a consequence, either of the chromosomal areas involved in the translocation may be a candidate region for a MD determining gene. To map precisely the translocation breakpoints, FISH analyses with YACs and PACs are in progress. The identification of the cryptic subtelomeric translocation segregating in this family is also of practical relevance, allowing us to offer prenatal diagnosis to the currently pregnant probands mother.

Chromosome 22q11.2 deletion in 6 infants with several clinical phenotypes. *V. Catala¹, E. Cuatrecasas¹, T. Vendrell², A. Plaja², M. Crespo¹, J. Colomer¹, C. Mediano², E. Sarret², A. Seres¹.* 1) Prenatal Genetics, Barcelona, Spain; 2) Unitat de Genetica, Hospital Materno Infantil Vall d'Hebron, Barcelona, Spain.

Chromosome 22q11.2 microdeletions are associated to different syndromes with a wide spectrum phenotypes. 50 infants with clinical features suggesting a possible 22q11.2 deletion were studied by FISH using TUPLE-1 probe (Vysis). Deletions were observed in 6 cases. Patient 1, aged 3, displayed minor facial features, hypocalcaemia and interrupted aortic arch. Both parents were cytogenetically normal. Patient 2 had dysmorphic face and a congenital conotruncal cardiac defect leading to a neonatal dead. The 22q11.2 deletion was observed in mosaic (89%). Patient 3 showed a retardation of growth and development and facial features, but had a normal heart. The 22q11 deletion was observed in the 60% of the analysed cells. Parents were cytogenetically normal. Patient 4 displayed an interrupted aortic arch. Both parents were normal. Patient 5 showed a truncus arteriosus and minor dysmorphic face. The mother was found to carry the 22q11.2 deletion. She displayed minor facial features and nasal speech and had a normal heart. The other son of the couple was studied by FISH showing no deletion. Patient 6 displayed immunopathy, dysmorphic face and a congenital cardiopathy. Both parents showed a normal karyotype.

Microdeletion of chromosome 17p13 in isolated lissencephaly sequence (ILS). *M.I. Melaragno, D. Brunoni, M.F. Galera.* Escola Paulista de Medicina, Sao Paulo Federal University, Sao Paulo, Brazil.

Classical lissencephaly is a brain malformation manifested by a smooth cerebral surface due to an arrest in the neuronal migration. It has been observed in association with the Miller-Dieker syndrome (MDS) or as isolated lissencephaly sequence (ILS). Literature shows that visible or submicroscopic deletions of 17p13.3 are found in about 90% of MDS patients whereas submicroscopic deletions occurring at the same locus are found in 10 to 30% of patients with ILS. A gene known as LIS1 was mapped to 17p13.3 and it has been responsible for classical lissencephaly. The present study focuses on clinical and cytogenetic aspects in ten patients with ILS. Cytogenetic analyses have been performed from lymphocyte cultures. Fluorescent in situ hybridization (FISH) was carried out on metaphase chromosomes using D17S379 Miller-Dieker region probe (Oncor) which hybridizes to specific sequences in 17p13.3. Chromosome 17q21 control probe (RARA) was used along with in order to facilitate chromosome identification. All the ILS patients showed apparently normal karyotypes using G-banding at 550-bands level resolution. However, FISH revealed deletion involving band 17p13 in one of them. The deletion was de novo, since signals on both chromosomes 17 in the patients parents karyotypes were present. No distinct difference was found between the clinical features of the patient with deletion and those with normal karyotype. Although FISH analysis has proved to be the most rapid and sensitive method of detecting deletion, it is not useful for the majority of the patients with ILS, as a considerable number of them (about 40%) presents point mutations or other subtle alterations of the locus LIS1. These findings emphasize the need to investigate not only the presence of molecular cytogenetic deletions but also the presence of mutations through molecular studies (FAPESP; CNPq, Brazil).

Adeno-Associated Virus rep/cap genes map to the active X chromosome and undergo episomal amplification in a HeLa based AAV producing cell line. *F. Lu*¹, *J.M. Wilson*², *N.B. Spinner*¹, *G. Gao*². 1) Division of Human Genetics, Dept of Pediatrics, Univ. of Penn School of Medicine, Philadelphia, PA; 2) Institute for Human Gene Therapy, Univ. of Penn School of Medicine, Philadelphia, PA.

Recombinant adeno-associated virus (AAV) is one of the most promising vectors for gene therapy. HeLa cells that contain the AAV rep/cap genes, are uniquely capable of serving as packaging cell lines for the AAV vector. We have studied the AAV packaging line, B50, a HeLa based cell line that has the AAV rep/cap genes stably integrated to understand the basis for this selective suitability of HeLa cells. Using fluorescence in situ hybridization (FISH) we mapped a single integrated rep/cap site to one of the two X chromosomes in the B50 genome. This was confirmed by dual-color FISH with a pericentromeric X chromosome probe. The rep/cap gene integrated into Xq26-27. Of interest, this integration site is in the vicinity of a global transcript activator (hSNF2L) that contains a Rep recognition sequence (RRS). This suggests that the Rep protein may play a role in this site-specific integration. In order to determine if the X chromosome containing the rep/cap genes was the active X, we carried out FISH to detect the replication pattern of the Xist gene. We studied 92 interphase nuclei and metaphase chromosomes. Twenty two percent of nuclei gave an asynchronuous pattern of Xist gene hybridization. This is consistent with a difference in the replication timing of this gene on the two homologues. All of those cells with an asynchronuous pattern had a doublet hybridization signal on the X chromosome without the rep/cap gene integrated. This is consistent with the X chromosome containing the rep/cap genes being the active X chromosome. We studied B50 cells after infection with adenovirus (AV) to analyze the effect on the rep/cap gene. Our results demonstrate that AV infection leads to the amplification of rep/cap gene episomally. Distinct images of the amplified rep/cap gene could be visualized in the cytoplasm 36 hours after AV infection. Rep/cap gene amplification may be a crucial requirement for AAV packaging.

Individual and chromosome specific differences in telomere length and age-related somatic cell aneuploidy frequencies: A twin study. *N.T. Leach, L.A. Corey, C.K. Jackson-Cook.* Human Genetics, VCU-MCV, Richmond, VA.

An increased frequency of sex chromosomal aneuploidy in lymphocytes is correlated with aging, especially in females. However, the frequency of age-related autosomal aneuploidy in various tissues is unclear. Also, the contribution that genetic and/or environmental factors make to age-related aneuploidy is not known. To gain this information, we determined the frequency of aneuploidy for a subset of chromosomes in lymphocytes (X,3,5,9,13,16,17 and 21) and buccal mucosa cells (X,17) using FISH. To date, we studied 10 female twin pairs ranging in age from 45 to 58 y.o. In lymphocytes, chromosomes X (3.9%) and 17 (3.2%) showed the highest level of loss, with chromosome 13 (0.6%) showing the lowest loss frequency. No clear association between chromosome size and its loss frequency was detected. Levels of chromosomal gain ranged from 1% (X chromosome) to 0.3% (chromosomes 9 and 17). The frequency of X chromosomal loss and gain was significantly lower ($p < 0.001$) in buccal mucosa cells compared to lymphocytes. In contrast, for chromosome 17 the difference in loss frequencies between tissues was less pronounced ($p = 0.03$), with no difference in chromosomal gain being seen ($p = 0.8$). Individual specific frequencies in somatic cell aneuploidy frequencies were also noted between females, with co-twins being more similar to one another than unrelated individuals. A potential association between aneuploidy frequencies and chromosome specific telomere length was determined for each chromosome arm using a semi-quantitative analysis of the intensities of a PNA pantelomeric probe. It may not be fortuitous that chromosome 17 (especially 17p) has been shown to have a small average telomere length and was also noted to have an increased level of somatic cell aneuploidy. This finding is of particular interest since loss of chromosome 17 has been observed in many types of cancer. In summary, we noted differences in the level of age-related aneuploidy between chromosomes and between individuals. Further studies to better define the role that genetic and environmental factors play in age-related aneuploidy should provide insight as to the clinical significance of this phenomena.

Fast-FISH using repeat sequence-depleted painting probes from microdissected DNA. *H. He¹, W. Huang¹, L. Scheller-Malin¹, X.Y. Guan².* 1) American Lab Technologies, Inc, Rockville, MD; 2) Department of Clinical Oncology, University of Hong Kong, Hong Kong, China.

There is currently an increasing demand by researchers and clinicians for high quality FISH painting probes that aid in the diagnosis of cancer and hereditary diseases. We have designed a novel method of removing repetitive sequences from microdissected probes resulting in products that are more specific and are easier to use. We named our repetitive sequence-depleted probes "ReSeD Probes". We tested our ReSeD probes of 5p, 9q, 12p, 15q and a few band specific probes in Fast-FISH. When used on metaphase chromosomes and interphase cells, the ReSeD probes produced strong, uniform, and specific hybridization signals with little background staining in only 30 minutes of hybridization. Dual-color Fast-FISH also produced comparable results. These new probes will make Fast-FISH a useful tool for the research and clinical community and allow faster turn around time for individual FISH cases.

Molecular and cytogenetic studies in Ambras syndrome. *M. Tadin*¹, *E.P. Braverman*⁵, *C-Y. Yu*¹, *J.A. Frank*¹, *F.A.M. Baumeister*³, *S. Cianfarani*⁴, *A.M. Christiano*^{1,2}, *D. Warburton*¹. 1) Department of Genetics and Development, Columbia University, New York, NY; 2) Department of Dermatology, Columbia University, New York, NY; 3) Dr. v. Haunersches Kinderspital der Universität München; 4) Department of Pediatrics and Public Health, University 'Tor Vergata', Rome, Italy; 5) School of Allied Health, University of Connecticut.

Ambras syndrome (AMS) is a unique form of congenital universal hypertrichosis. In patients with this syndrome the whole body is covered with fine, long vellus hair, except for areas where no hair normally grows. There is accompanying facial dysmorphism and abnormalities of the teeth, including retarded first and second dentition, and absence of teeth. No metabolic or endocrine defect has been detected. Multiple affected family members suggest a genetic basis for the syndrome. An inversion of chromosome 8 has been found in two isolated cases. These inversions have a breakpoint in band q22 in common, suggesting that this region (8q22) contains the gene which causes AMS. Using chromosomal FISH analysis we have narrowed the breakpoint interval to about 500 kb in one of the patients. We also performed loss of heterozygosity (LOH) analysis to check for possible microdeletions that are not evident at the cytogenetic level. A deletion of about 1 Mb was detected in the other patient in the vicinity of the previously defined 500 kb inversion interval. We are currently saturating the region with additional microsatellite markers to determine the borders of the deleted interval. We anticipate that these combined approaches will further narrow the interval and lead to the identification of the Ambras syndrome gene.

A case with an extra large inverted duplicated chromosome 15. *S.-D. Cheng*¹, *S.-P. Lin*². 1) Department of Anatomy, College of Medicine, Chang Gung University, Kweishan, Taiwan; 2) Division of Genetics, Department of Pediatrics, MacKay Memorial Hospital, Taipei, Taiwan.

The proximal region in the long arm of chromosome 15 (15q11q13) has been known to be related to the Angelman syndrome (AS) and Prader-Willi syndrome (PWS). The increase of the copy number of the region, depending on the size, may also create abnormalities to the phenotype. With fluorescence *in situ* hybridization (FISH) method combined with quantitative Southern analyses, we detected a proband with an extra large inverted duplicated chromosome 15 (inv dup(15)). Under DAPI staining an extraordinarily long segment is revealed between two centromeres of the inv dup(15). We tested with FISH with lambda clones of 34-10 (D15S9), 116 (GABRB3) and IR10-1-45 (D15S12) individually. Each of the three probes revealed four copies of the signals on each sister chromatid of the inv dup(15). Together with two copies of each locus on the normal chromosomes 15, the proband has six copies totally in her genome. The quantitative Southern analyses with PhosphorImager indicated that the copy number of p3-21 (D15S10) locus of proband is 2.27 times, and that of p189-1 (D15S13) is two-fold than that of a normal female after normalization with probe 87-15 (DXS164). The proband at her age of 12 featured short stature, hypertelorism, upward slant of palpebral fissures, thin upper lips, mildly dysplastic ears, and simian crease of right hand. She had developmental delay with mental retardation and poor language ability. Other than emotional instability and hyperactivity, she also had neurofibromatosis type I, which is inherited from her mother.

An *In Situ* Hybridization-Based Strategy to Detect Neuronal Patterns of Genomic Imprinting in the Autism

Candidate Region of Chromosome 15q11-q13. R.A. Kesterson¹, E.L. Nurmi¹, D.K. Johnson², J.S. Sutcliffe¹. 1) Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN; 2) Mammalian Genetics and Development Section, Oak Ridge National Laboratory, Oak Ridge, TN.

Reciprocal patterns of genomic imprinting are involved in the etiology of genomic disorders associated with human chromosome 15q11-q13. Paternal deletions of 15q11-q13 are associated with Prader-Willi syndrome, while maternal deletions of a common region are associated with Angelman syndrome. Interstitial duplications or triplications using similar homologous breakpoints, or "duplicons", and inverted duplications utilizing more distal breakpoints are associated with autism-spectrum phenotypes, possibly in a maternal-specific manner. An autism candidate region has been suggested based on the duplications, as well as linkage and linkage disequilibrium data in a common area. Given the possible involvement of genomic imprinting in the etiology of autism susceptibility in the chromosome 15 region, we are assessing positional and functional candidate genes in this region for neuronal patterns of genomic imprinting using an *in situ* hybridization-based strategy for their murine orthologs. Mice with large deletions encompassing the p locus are hemizygous for genes within the autism candidate region. Using mice with reciprocal maternal or paternal deletions, or controls, we hybridized brain sections with probes corresponding to a cluster of GABA_A receptor subunit genes (*Gabrb3*, *Gabra5*, and *Gabrg3*), and the p locus. Our initial studies have revealed that these genes do not show complete allele-specific silencing within the brain. We are currently exploring the possibility of more subtle allelic differences in expression. It is feasible that genes within this region may show complex imprinting effects as evidenced by maternal-biased expression of *Ube3a* within certain brain regions. This experimental strategy provides greater sensitivity to detect genomic imprinting in this region compared to northern-based approaches which may not detect subtle patterns of parental-specific expression.

Molecular analysis of Severe Mental Retardation associated 16p-chromosomal breakpoint. *K. Bhalla, H. Eyre, A. Gardner, G. Kremmidiotis, G.R. Sutherland, D.F. Callen.* Department of Cytogenetics and Molecular Genetics, The University of Adelaide, Women's & Children's Hospital, Adelaide, Australia.

Identification of families with mentally retarded males has led to the mapping and cloning of a number of X-linked recessive loci and relevant genes for mental retardation. However, there has been little progress in the identification of autosomal genes for mental retardation. We have exploited a *de novo* balanced translocation, 46,XY,t(1;16)(q12;p13.3) in a patient with severe intellectual retardation. We have generated a somatic cell hybrid containing the der(16) of this translocation, designated as CY196. Since the chromosome 1 breakpoint was shown to be within 1q heterochromatin, we propose that this translocation either disrupts a gene on chromosome 16 or alters expression of a gene in the near vicinity by a position effect of the chromosome 1 heterochromatin. Preliminary work in the vicinity of the breakpoint identified *C16orf5*, a novel proline rich gene, highly expressed in the brain. The YAC pulse field map of the region placed this gene distal to CY196. A PAC/BAC contig was then constructed across the chromosome 16 breakpoint. FISH analysis with these clones to the metaphase chromosome identified a PAC clone spanning the breakpoint. The PAC was sequenced and analysis indicates that the region surrounding the breakpoint is repetitive with very few matches to expressed sequences (ESTs) or computer predicted exons. *C16orf5* is the closest gene to the breakpoint and is highly expressed in the brain. Quantitative RT-PCR analysis suggests that the expression of the *C16orf5* transcript is reduced in the patient. We propose that the mental retardation of the translocation patient is the result of reduced expression of this brain specific transcript due to the position effect of the adjacent translocated chromosome 1 heterochromatin. Mutation analysis in the candidate gene will now be carried out to show its involvement in mental retardation.

Simultaneous Detection of Three Different Chromosomes in Multi-PRINS Using only two types of labeled nucleotides. Yan J, Bronsard M, Drouin R. Department of Medical Biology, Division of Pathology, Faculty of Medicine, Universit Laval, Hpital Saint-Franois d'Assise, CHUQ, Qubec, Qubec, Canada. *J. Yan, M. Bronsard, R. Drouin.* Dept Medical Biol, Laval Univ, CHUQ Hop.St-Francois d'Assise, Quebec, Quebec, Canada.

In multi-PRINS technique, the blocking step using ddNTP incorporated by a DNA polymerase is an important procedure that can block the free 3-end generated in the last PRINS reaction, thus avoiding the next PRINS reaction to use it as a primer to perform a spurious elongation at non-desired sites. However, in our tests, omission of the blocking step never affected the correct identification of two chromosomes because the signals from the second PRINS reaction site always showed the pure original color. Taking advantage of the color mixing and creating a new color, we successfully performed a multi-PRINS technique to obtain a third color for simultaneous detection of three chromosomes in the same cell. By arranging the incorporation order of the labeled nucleotides either in bio-dig-bio or in dig-bio-dig, two most common labeled nucleotides, biotin- and digoxigenin-dUTP, were alternatively incorporated in the newly synthesized DNA strand during three sequential PRINS reactions. Two detection systems were coupled with the incorporation order: 1) avidin-fluorescein mixed with anti-dig-rhodamine was used for bio-dig-bio incorporation order and 2) anti-dig-fluorescein mixed with avidin-rhodamine was used for dig-bio-dig incorporation order. Three different chromosomes can be identified as yellow, red and green color signals, respectively. We measured the relative intensity of the two original red and green color elements. The results showed that a red signal can contain up to 38% of green color and still appears red, whereas a fluorescent signal must contain at least 43% of green color to generate the yellow color. A green signal can contain up to 12% of red color and still retain its green color. The entire procedure could be completed in less than 90 minutes because the blocking step is omitted. We believe that this multi-PRINS technique is a practical and efficient way to simultaneously detect three or more chromosomes.

Towards a Full Karyotype Screening of Interphase Cells: 'FISH and Chip' Technology. *H.U. Weier¹, J. Fung^{1,2}, S. Munné³, R.A. Lersch¹, H.B. Hsieh¹, X.N. Chen⁴, J. Korenberg⁴, R.A. Pedersen².* 1) Lawrence Berkeley Natl. Laboratory, Berkeley, CA 94720; 2) Department of Ob/Gynecology and Reproductive Sciences, UCSF, CA 94143-0720; 3) St. Barnabas Medical Center, Livingston, NJ 07052; 4) Medical Genetics Birth Defects Center, UCLA, CA 90048.

Translocations carriers have an elevated risk of producing aneuploid germ cells due to disturbed homologue pairing. The resulting numerical chromosome aberrations lead to spontaneous abortions, stillbirth or severe deficiencies and disease. Our laboratories develop technologies to study the chromosomal composition of single interphase cells, e.g., blastomeres. Our work is facilitated by access to large insert genomic DNA libraries, physical maps or collections of cytogenetically mapped DNA probes. Most breakpoint-specific probe sets for interphase cell analyses of patient cells are prepared within a few weeks. Once optimized, these probe sets allow to rapidly determine the number of normal and derivative chromosomes in somatic or germ cells from the carriers as well as their offspring. So far, however, these procedures failed to produce the desired increase in pregnancy rates. Our concern is that gain or loss of other chromosomes due to impaired homologue pairing remains undetected in these assays. Most fluorescence in situ hybridization (FISH) studies using filter-based microscope systems were limited to simultaneous use of 3-4 differently labeled probes. Spectral Imaging (SI) now allows an investigator to discriminate an increased number of probes by exciting fluorescence over a broad spectral range and recording the emission spectrum with an interferometer. SI allows to interrogate a much larger number of targets thus producing a more comprehensive picture of the chromosomal composition of cells. Using mostly YAC/BAC probes for analyses of blastomeres, we are building panels of probes to simultaneously score 10 chromosomes. Further increases in the number of probes are complicated by spatial overlap of chromosome domains. We are presently investigating a complementing strategy, in which the DNA probes are immobilized and DNA from the embryonic cells is hybridized to such a DNA Chip in a quantitative manner.

Interphase FISH analysis on uncultured cells for prenatal diagnosis. *H. Bruyere, S. Masui, D. Dowsley, D.E. McFadden, T. Pantzar, E. Separovic, F.J. Dill, D.K. Kalousek.* Cytogenetics Laboratory, Department of Pathology, Children's & Women's Health Centre of BC, Vancouver, BC, Canada.

Interphase fluorescence in situ hybridization (FISH) on uncultured amniocytes with a commercially available cocktail of specific probes for chromosomes 13, 18, 21, X and Y is currently being assessed for its use as an alternative or an adjunct to conventional cytogenetics in prenatal diagnosis. It is helpful for rapid confirmation of the most common aneuploidies when the patient is near the legal limit for termination of pregnancy or in late pregnancy when decisions regarding the delivery of fetuses with ultrasound abnormalities are made. FISH analysis is performed along with conventional cytogenetic analysis on amniotic fluid. Among 250 cases analyzed during a 28-month period, 38 common aneuploidies and 3 triploidies were detected. In 9 pregnancies with chromosomal structural abnormalities, FISH showed an abnormal result in 3 cases: 1) An extra derivative chromosome 18 presented as a trisomy 18. 2) An unbalanced robertsonian translocation was correctly interpreted as a trisomy 21. 3) Two cell lines involving missing X chromosome and a rearranged X chromosome were identified. The 6 remaining cases involved an interstitial deletion of chromosome 17q, three 22q11 microdeletions, a paternally inherited balanced translocation between chromosomes 14 and 21 and a derivative chromosome 22 resulting from the abnormal segregation of a maternal balanced translocation previously undiagnosed. Our results show that interphase FISH analysis on uncultured amniocytes is a valuable tool for the rapid detection of the most common aneuploidies. Although this test is not designed to detect structural rearrangements, it may show an abnormal result when the structural abnormality involves one of the 5 chromosomes tested.

Mosaic triple trisomies isolated to amniocytes. *T. Creeden¹, D. Day-Salvatore¹, L. Sciorra¹, S. Finkernagel¹, T. Kletz¹, E. Guzman².* 1) Division of Clinical Genetics, UMDNJ-Robert Wood Johnson Medical School and Saint Peters University Hospital, New Brunswick, NJ; 2) Division of Maternal Fetal Medicine, UMDNJ-Robert Wood Johnson Medical School and Saint Peter's University Hospital, New Brunswick, NJ.

We report the prenatal detection of a mosaic triple trisomy, 46,XX/49,XX,+13,+17,+20, in amniocyte culture. Repeat amniocentesis demonstrated the same mosaic triple trisomy results while placental biopsy and percutaneous umbilical blood sampling yielded normal non-mosaic female karyotypes. Targeted level II ultrasonography revealed normal fetal anatomy and appropriate fetal growth. A healthy female infant was delivered at term; postnatal chromosome analysis of lymphocytes and fibroblasts were normal. We postulate that this triple trisomy may be confined to the fetal kidneys or bladder, as this would explain the presence of the trisomic cells in amniotic fluid only. Postnatal renal ultrasounds were reported as normal. Urine sediment chromosome analysis was attempted, but failed to yield results. This is the second reported case of mosaic triple trisomy; the previous case was 46,XY/49,XY,+13,+20,+21 and resulted in a phenotypically and karyotypically normal live born infant. Trisomy 20 has been confined to the placenta or other fetal organs, such as kidney or gut, and results in a normal outcome in the majority of cases. This case provides further evidence that mosaic triple trisomies, involving chromosome 20, may be an extension of the benign isolated trisomy 20 mechanism.

Clinical associations of women experiencing recurrent spontaneous abortion (RSA) and exhibiting skewed X chromosome inactivation (XCI). *C.L. Beever¹, M.D. Stephenson^{2,3}, V. Popovska², R. Jiang¹, K.K. Sangha¹, K.*

Ochnio¹, C.J. Brown¹, W.P. Robinson^{1,3}. 1) Dept. of Medical Genetics; 2) Dept. of Obstetrics & Gynaecology, Univ British Columbia; 3) Children's & Women's Hospital & Health Centre of B.C., Vancouver, BC, Canada.

RSA, defined as 3 or more consecutive losses under 20 weeks gestation, is estimated to affect 1-2% of couples trying to have a family. Skewed XCI, scored as $\geq 90\%$ inactivation of one X allele, is significantly increased in women experiencing RSA. To identify the cause of this association, XCI status in blood was determined using a methylation sensitive assay at the androgen receptor locus and correlated with clinical features in RSA women. 19% of the RSA women (n=113) were found to have skewed XCI compared to 5% of controls (n=111) (p=0.001). Preliminary studies of buccal samples from a subset of the RSA women showed that skewed XCI can be present in both blood and buccal cells. If RSA and skewed XCI were the result of women being carriers of X-linked lethal mutations, we would expect an excess of male abortuses and an excess of female livebirths in those women with skewed XCI. However, 2 of 3 karyotyped abortuses and 7 of 10 livebirths were male. If RSA and skewed XCI were the result of maternal germline trisomy mosaicism, we would expect an excess of age-independent aneuploid losses from RSA women with skewed XCI. Although 10 of 14 karyotyped abortuses from the skewed XCI women were aneuploid as compared to 30 of 73 abortuses from the non-skewed XCI women (p=0.02), increased maternal age was associated with aneuploid losses in both the skewed and non-skewed groups of women with RSA. Of the 17 couples with RSA and skewed XCI who completed clinical investigations, 8 were classified as having autoimmune-associated RSA, 8 with unexplained RSA and 1 with anatomical-associated RSA. Although preliminary data show this is not statistically significant compared to the non-skewed group, the trend towards an increased rate of autoimmune-associated and unexplained RSA couples is of interest. Further studies will clarify these associations and assist in the management of couples with this complex reproductive problem.

X/Autosomal translocations in the Xq region associated with premature ovarian failure fall both within and outside genes. *L.M. Herrera¹, S. Mumm², A. Forabosco³, P.W. Waeltz¹, L. Ruini³, R. Nagaraja¹, T. Esposito³, M. Rocchi⁴, D. Schlessinger¹.* 1) Laboratory of Genetics, National Institute on Aging, Baltimore, MD; 2) Division of Bone and Mineral Disease, Washington University School Medicine and Barnes-Jewish Hospital, St. Louis, Missouri; 3) Department of Morphology and Legal Medicine Sciences, University of Modena and Reggio-Emilia, Modena, Italy; 4) Istituto di Genetica, Bari, Italy.

Premature ovarian failure (POF), which affects 1% of women, is characterized by a cessation of ovarian function before the age of 40 years. POF has been linked to X-chromosome abnormalities, including X/autosome balanced translocations. Mapping of breakpoints has shown that they tend to concentrate in a large "critical region" (Xq13-Xq26), that is inferred to be required for the maintenance of ovarian function and normal reproductive lifespan. To account for X involvement in POF, one model predicts that breakpoints interrupt specific genes involved in ovarian development; a second model predicts that defects in meiotic pairing provoked by the rearrangements in X chromosome result in excessive apoptosis of follicles. For the second model, breakpoints might fall inside or outside of genes. Three translocations located at the borders of the critical region were analyzed (one in Xq13.3, and two in Xq26). Patient chromosomes were isolated in somatic cell hybrids, breakpoints were mapped, and sequence analysis was carried out. The Xq13.3 breakpoint falls in a region that is essentially totally comprised of repeat sequences, with a low GC content and no gene within 40kb in either direction. One breakpoint in Xq26 maps in a highly transcribed region, and when fully analyzed, interrupts a gene that encodes a widely-expressed member of the ETS transcription factor family. The third breakpoint falls in uncloned DNA, though in a gene-poor region. The analyses suggest that POF can result from breaks both within and outside of genes, increasing the possibility that a disturbance of X chromosome dynamics, perhaps related to X-inactivation, may be involved in the etiology of the condition.

Satellite-like Projection of Chromatin - a possible marker for anticipation of fragile sites, detected in a case of Fanconi Anemia. *P.F. Madon, A.S. Athalye, D.J. Naik, V.C. Sarangdhar, F.R. Parikh.* Dept.of Assisted Reproduction & Genetics, Jaslok Hospital, Bombay, India.

A satellite-like projection of chromatin situated along the length of the outer margin of some chromosomes, has occasionally but consistently been observed during routine cytogenetic analysis. This marker is more commonly seen while screening for fragile sites, when blood is cultured in a low folic acid medium. It has also been observed in the fuzzy outline of some chromosomes in leukemia patients. Its appearance is similar to the stalk and satellite of acrocentric chromosomes. On further analysis it was observed that the location of this marker was mainly confined to G-light band regions and corresponded with fragile sites. However no visible chromatid gap was seen. Recently, while analysing the results of the Mitomycin C (MMC) chromosome stress test in a 2 year old boy from Bhopal suspected to have Fanconi Anemia, this satellite-like marker was noticed in 2% of 400 metaphases. In some cells, the location of this marker was at the same site at which chromatid breaks were observed. The child had congenital bilateral syndactyly of all digits on the hands and distal triphalangeal thumbs. MMC tests showed 88% breaks and 10% radials in cultures at a final concentration of 80ng/ml MMC as compared to 38% breaks and 2% radials in his parents. He was diagnosed as having Fanconi Anemia though he did not have other clinical manifestations at this age except a low Hb count of 10g/dl. We hypothesize that this marker could help in early identification of hotspots of fragile sites even before the unstained gap is visible. This could have important implications as fragile sites are known to play an integral role in tumorigenesis as they often occur at the site of cellular proto- oncogenes. Further studies using different fragile site inducing agents, CGH and scanning electron microscopy are indicated. Similar markers have been reported to be linked with DNA amplification of oncogenes, nucleolar organising regions and double or single minutes. Chromosome microdissection if possible and molecular analysis may help to characterize this marker and develop a means to anticipate cancer. >>>>.

Complex chromosomal rearrangement and review of familial cases. *G.S. Sekhon¹, M.S. Williams², K.J. Thompson¹, M.K. Sharifi¹.* 1) Div Clinical Gen, Waisman Ctr, Univ Wisconsin, Madison, WI; 2) Dept. of Pediatrics, Gundersen Lutheran Medical Center, LaCrosse, WI.

A familial case of complex chromosomal rearrangement (CCR) was ascertained through a male newborn infant with respiratory distress and multiple congenital anomalies including wide sagittal suture, flat occiput, bifid uvula, short thumbs, clinodactyly and camptodactyly of the fifth fingers, bilateral hip dislocations, small penis, undescended testes, and a two vessel cord. Neurological examination identified an unusual cry and abnormalities of muscle tone. CT of the head demonstrated a Dandy-Walker variant. No renal or cardiac anomalies were present. The mother had a balanced reciprocal translocation involving chromosome 1, 4, and 9. The karyotype is: 46,XX,t(1;9;4)(q44;q32;q33). The malsegregation of the CCR resulted in partial duplication of chromosome 4 and 9 and a tiny deletion of 1q terminus resulting in karyotype: 47,XY,der(1)t(1;9;4)(q44;q32;q33),+der(9)t(1;9;4)(q44;q32;q33)mat. A review of 40 familial cases of CCRs led to the following: 1) familial CCRs have fewer chromosomes involved and fewer breakpoints than de novo CCRs, 2) there is an excess of balanced female carriers among the offspring of index carriers, 3) familial transmission is usually through female carriers although the origin of de novo abnormality is frequently paternal, 4) meiotic segregation resulting in abnormal liveborn infant is usually due to adjacent 1 segregation, followed by 4:2 segregation.

Gene expression analysis of developing mouse utricle by oligonucleotide array and implication in deafness gene identification. Z. Chen^{1,2}, K. Karimi³, D. Zhang^{1,4}, M. Rivolta⁵, R. MacDonald¹, M. Holley⁵, J. Corwin³, D. Corey^{1,2,4}.
1) Neurology Dept, Massachusetts General Hosp, Boston, MA; 2) Dept. of Neurobiology, Harvard Medical School, Boston, MA; 3) Dept. of Otolaryngology-HNS and Neuroscience, University of Virginia School of Medicine, Charlottesville, VA; 4) Howard Hughes Medical Institute; 5) Dept. of Physiology, School of Medical Sciences, University of Bristol, UK.

Microarray technology utilizes a massively parallel approach to study gene expression at the global level. This approach is particularly suited to investigate the expression pattern of the inner ear, where limited tissue and inaccessibility has traditionally hampered study on its development and function. The utricle is one of two vestibular organs in the inner ear that detects gravity and linear acceleration of the head. With a relatively simple organization, it is a useful model for other hair-cell organs such as the cochlea and semicircular canals. Using oligonucleotide array technology, we studied the gene expression profile of the mouse utricle during developmental stages critical for maturation of hair cell function. Oligonucleotide array analysis is sufficiently sensitive to detect genes expressed in the hair cells (~1,000 hair cells per utricle). Cluster analysis revealed genes involved in pathways important to the development and function of the utricle. Enzymatic and pharmacological approaches were employed to select different cell populations for oligonucleotide analysis, which allows the identification of cell-type-specific pathways in the multicellular environment. Cell lines derived from mouse utricle showed expression profiles similar to that of utricle, suggesting that it can be used to study genes specifically expressed in the utricle. Most known deafness genes were found in the clusters representing the mature utricle, suggesting that these clusters may contain additional genes for inherited hearing loss. The ability to cluster disease genes in a particular tissue will have an important implication for disease gene identification in general.

SLC5A3 GENE EXPRESSION REGULATES FETAL MYO-INOSITOL LEVELS. *R. Buccafusca, S. Wu, J. Golden, K. McVeigh, G. Berry.* Pediatrics, University of Pennsylvania, Philadelphia, PA.

A Na⁺/myo-inositol cotransporter such as the SLC5A3 protein is responsible for Na⁺ and energy dependent concentrative uptake of myo-inositol in certain mammalian tissues. The synthesis of membrane phosphoinositides such as phosphatidylinositol-4,5-bisphosphate, phosphatidylinositol-3-phosphate and phosphatidylinositol-anchored proteins is dependent on an adequate concentration of cellular myo-inositol. During fetal life, myo-inositol is maintained at much higher concentrations in blood and spinal fluid than in the adult mammalian organism. In order to assess the importance of the SLC5A3 gene in fetal-placental homeostasis of Ins, we generated SLC5A3 (-/-) and (+/-) mice by targeted gene inactivation and studied the effect on gene expression and fetal Ins levels. The SLC5A3 (-/-) mice die shortly after birth, manifest no SLC5A3 mRNA and have a 80-90% reduction in whole fetal Ins levels. The gene is highly expressed in fetal and placental tissues. Carriers demonstrate intermediate tissue Ins levels and SLC5A3 mRNA levels that persist into adult life. The homozygous SLC5A3 null mutation is lethal probably because of severe Ins deficiency as the SLC5A3 gene is essential for maintenance of the Ins concentration gradient in the fetal-placental unit. The consequences of SLC5A3 underexpression in the carrier state remain to be determined.

Expression pattern of the NOGGIN gene during human bone development fits with the clinical spectrum of proximal symphalangism and multiple synostosis syndrome but not with spondylocarpotarsal synostosis. *J. Augé¹, V. Cormier-Daire¹, J. Amiel¹, S. Audollent¹, J. Martinovic¹, F. Encha-Razavi¹, D. Esnault¹, S. Lyonnet¹, A. David², A. Munnich¹, M. Le Merrer¹, M. Vekemans¹, T. Attié-Bitach¹.* 1) Dep de genetique INSERM-U393, Hopital Necker-Enfants Malades, Paris, France; 2) Service de génétique médicale, CHU Hopital Mère Enfant, Nantes, France.

Recently NOGGIN mutations have been identified in two autosomal dominant disorders both characterized by multiple joint fusion: proximal symphalangism (SYM1) and multiple synostose syndrome (SYN1). Interestingly, the *noggin*^{-/-} mutant mice present joint vertebral body fusion and synostoses of proximal large joints, both features being reminiscent of spondylocarpotarsal synostosis syndrome or synspondylism in human. Therefore, in order to assess the role of NOGGIN in joint fusion syndromes, we sequenced the NOGGIN gene in 5 cases of SYM1, SYN1 or synspondylism and performed in situ hybridization studies of NOGGIN during human development. A 12 bp NOGGIN deletion was identified in a SYN1 case and a missense mutation (P35S) was found in a SYM1 case also presenting with complete absence of distal phalangia. Accordingly, the NOGGIN gene was expressed in cartilage of all bones examined, namely phalangeal, carpal, tarsal, rib and vertebral bones. NOGGIN was also strongly expressed in perichondrium of distal phalanges, medial carpal bones and cubitus, but not in vertebral perichondrium. Consistent with these results, we failed to find any NOGGIN mutation in three patients with synspondylism. This study confirms the involvement of NOGGIN in SYN1 and SYM1 and suggests that NOGGIN is not associated with synspondylism in humans.

Neuronal migration defects in Lis1 mutant mice are accompanied by abnormalities in cell Proliferation and survival^{Lis1}. M.J. Gambello^{1,2}, A. Wynshaw-Boris¹. 1) Department of Pediatrics, UCSD School of Medicine, San Diego, CA; 2) HHMI Physician Fellow, UCSD, San Diego, CA.

Haploinsufficiency of the LIS1 gene plays a major role in the etiology of two human neuronal migration disorders characterized by classical lissencephaly: Miller-Dieker syndrome and isolated lissencephaly sequence. Mice heterozygous for a Lis1 null allele (HET) exhibit a cell autonomous in vivo and in vitro neuronal migration defect. To understand the pathogenesis of classical lissencephaly, a developmental analysis was done in HET and compound heterozygous mice (CH) containing a null and a hypomorphic allele. BrdU birthdating in Lis1 mutant mice demonstrate a slowing of neuronal migration proportional to Lis1 reduction. The preplate of Lis1 mutant mice is split into the marginal zone and subplate by invading cortical plate neurons, however in the CH the subplate remains diffuse, suggesting that some neurons failed to migrate out of this zone. By E15.5 CH brains are smaller than wildtype brains suggesting a defect in proliferation and/or cell survival. Analysis of neural precursor proliferation of E15.5 embryos by BrdU pulse labeling reveal a 20 and 40% reduction in absolute number of proliferating cells in HET and CH mice respectively, but a similar mitotic index in all three genotypes. Finally, histologic analysis reveals more pyknotic nuclei in embryonic and postnatal CHs suggesting increased apoptosis. To further assess the possibility of cell cycle defects, mitotic figures were identified by immunohistochemistry. In wildtype brains most mitoses are seen along the ventricular lumen with few in the subventricular zone. The CH shows fewer neuroblasts in M phase along the ventricular lumen, and more M phase cells scattered throughout the thinner ventricular zone. This may represent a defect of interkinetic nuclear migration and/or be reflective of cell cycle dysregulation. These development analyses of Lis1 mutants demonstrate a tripartite role for Lis1 in the pathogenesis of classic lissencephaly involving delayed neural migration, cell proliferation, and apoptosis. *LIS1Lis1Lis1Lis1*
Lis1Lis1.

Ectopic over-expression of the dosage dependent gene, ZIC2, in transgenic mice results in severe CNS malformation. *L.Y. Brown, S. Brown.* Obstetrics and Gynecology, Columbia University, New York, NY.

Zic2 is zinc finger transcription factor which is one of the vertebrate homologues of the *Drosophila* odd-paired or *opa* gene. That Zic2 has a critical role in CNS development has been demonstrated by the fact that heterozygous mutations in humans result in holoprosencephaly (HPE) and diminished gene dosage results in a similar phenotype in mice. In addition, chromosome 13 q32 deletions which include ZIC2 result in severe brain malformations. All of these observations demonstrate that brain development is exquisitely sensitive to the level or dosage of Zic2 protein present, although the developmental pathway in which Zic2 functions is unknown. Because Zic2 is dosage sensitive, we sought to test the hypothesis that increased levels of Zic2 protein during development may also result in brain malformation and that increased Zic2 dosage may be part of the basis for the developmental defects caused trisomy 13 and distal chromosome 13 duplications. To this end, we placed the ZIC2 cDNA under the regulation of the enhancer elements of the neurofilament protein, nestin and introduced this construct into mouse oocytes by pronuclear injection. Injected pronuclei were transferred to foster mothers in order to generate transgenic mice. Foster mothers were sacrificed at day 12.5 and day 14.5 of gestation and embryos were inspected grossly and analyzed by serial sectioning and in-situ hybridization. All embryos which expressed the transgene were multiply malformed. Malformation ranged from exencephaly in the most severe cases to distortions of the spinal cord and forebrain structures in less severe cases. All of the malformations seen were likely to be lethal. Analysis of transgenic embryos showed an over abundance or over proliferation of neural tissue in response to transgene expression, whereas the basic patterning of CNS structures, in general, remained intact. This result suggests that ZIC2 has a role in proliferation and or maintenance of specific pre-neuronal cells of the CNS. Current experiments are directed towards a more refined model of ZIC2 overdosage and towards and understanding of the pathway through which ZIC2 acts.

opa.

Comparative expression of the SHH, GLI3 and JAG1 genes in normal and neural tube defects human embryos.

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SHH, GLI3 and Jagged1 (JAG1) are known to be important determinants of early development in several species. In humans their mutations are associated respectively with holoprosencephaly, Greig, Pallister-Hall and Allagille syndromes. Here we analysed their central nervous system (CNS) expression pattern in normal as well as in 5 neural tube defects (NTD) human embryos. In normal embryos (CS 12 to 21), GLI3 is expressed in the dorsal part of the neural tube in an opposite manner to the SHH gene which is expressed in the ventral part of the CNS and in the floor plate. The JAG1 gene is expressed in a ventral region of the neural tube flanking the floor plate, in a dorsal region flanking the roof plate as well as in two middle strips at and below the sulcus limitans. In the cranial region of the NTD embryos, additional domains of expression of the GLI3 and JAG1 gene are observed. In the thoracic region, where several expression domains of the SHH gene are observed, GLI3 expression is abnormally detected in the floor plate. Also additional domains of JAG1 expression are still observed in this region. In the caudal region SHH gene expression is displaced suggesting a vertical floor plate. A symmetrical GLI3 gene expression is observed on either side of the closed neural tube but an asymmetrical (or absent) pattern of expression is observed in the open one. Further studies are necessary to identify genes that are differentially expressed between normal and NTD embryos. For example, large-scale gene expression studies using microarray techniques should contribute to our understanding of the pathogenesis of neural tube defects in humans.

Novel polymorphisms in the upstream region of VIPR2 gene suggest a possible role in gastrointestinal problems and stereotypical behaviors in autism. *E. Asano¹, H. Kuivaniemi², M. Huq¹, G. Tromp², M. Behen¹, R. Rothermel¹, J. Herron¹, D.C. Chugani¹.* 1) Children's Hosp. of Michigan, Wayne State Univ., 3901 Beaubien St., Detroit, MI; 2) Center for Molecular Medicine and Genetics, Wayne State Univ., Scott Hall 3106, 540E. Canfield Ave., Detroit, MI.

We investigated the vasoactive intestinal peptide receptor type 2 (VIPR2) gene as a candidate gene for autism because: (1) VIPR2 gene is mapped near the region on 7q with suggested linkage to autism, (2) VIPR2 is expressed in embryonic brain, (3) VIP has neuroprotective actions, and (4) many autistic children have gastrointestinal problems. We searched for mutations in the VIPR2 gene in autistic patients and identified a novel seven-nucleotide deletion in the promoter region. We also found nine novel single-nucleotide polymorphisms (SNPs): four in the promoter region, one in the 5' UTR, and four in the coding region, none of which changed an amino acid. Three polymorphisms in the upstream region were studied in detail and there was no significant difference in the frequencies between the autistic group (N=15) and unrelated controls (N=52). Autistic subjects who were heterozygous for the seven-nucleotide polymorphism, however, had gastrointestinal problems more frequently than those with homozygous insertion or deletion genotype (Fishers exact test, $p=0.028$). Conversely, autistic patients with GG genotype for the A-G SNP of the 5' UTR had gastrointestinal problems more frequently than those with AG or AA genotypes ($p=0.030$). Moreover, there was a correlation between the genotypes for the C-T SNP of the promoter region and the severity of stereotypical behavior as ranked by Gilliam Autism Rating Scale (Spearman's rank order test, $p=0.039$). These preliminary results suggest that VIPR2 may be a modifier locus for autism altering the presence or severity of gastrointestinal problems and stereotypical behaviors.

Aristaless Related Homeobox (ARX) Gene is Expressed in a Subset of Neuronal Precursor Cells and Post-Mitotic Neurons in Human Fetal and Adult Brain. *R. Ohira*¹, *Y.-H. Zhang*², *W. Guo*², *K. Dipple*², *S.L. Shih*², *J. Doerr*³, *B.-L. Huang*², *L.J. Fu*⁴, *A. Abu-Khalil*⁴, *D.H. Geschwind*⁴, *E.R.B. McCabe*². 1) Human Genetics; 2) Pediatrics; 3) Microbiology and Molecular Genetics; 4) Neurology, UCLA, Los Angeles, CA.

Arx is a homeobox-containing gene with a high degree of sequence similarity between mouse and zebra fish (Miura et al, *Mech Dev* 65:99, 1997). Arx is expressed in the forebrain and floor plate of the developing central nervous systems (CNSs) of these vertebrates and in the presumptive cortex of fetal mice. Our goal was to identify genes in Xp22.1-p21.3 involved in human CNS development. Our in silico search for candidate CNS developmental genes noted that annotation of a human Xp22 PAC (RPC11-258N20) sequence (GenBank No. AC002504) identified putative exons consistent with an Arx homologue in Xp22. Northern blot analysis showed that human ARX was expressed in fetal and adult brain, and we also observed increased levels of expression in placenta and in adult heart, skeletal muscle, liver and kidney. In situ hybridization with an ARX anti-sense cRNA probe onto human brain sections at various developmental stages (fetal-adult) showed highly restricted expression patterns that differed from those reported in mouse. Embryonic expression was highest in neuronal precursors in the germinal matrix and a subset of post-mitotic neurons in the cortex and hippocampus. ARX marked a discrete subset of cortical neurons in the adult. The expression pattern suggests that ARX is involved in the differentiation and maintenance of specific subsets of neuronal phenotypes in the human CNS. We also mapped the murine Arx gene to the mouse genome using a mouse/hamster radiation hybrid panel to determine whether Arx and ARX are orthologues or simply members of the same gene family. Arx mapped to the murine X chromosome in the region containing Dmd, Gyk and Zfx and syntenic with the ARX locus. We conclude that ARX is the human orthologue of murine Arx and is an Xp22 gene involved in human fetal brain development. We speculate that ARX is a candidate gene for X-linked mental retardation and/or structural brain abnormalities.

Abnormal signalling pathway caused by FGFR 3 mutations in the cartilage of Achondroplasia and Thanatophoric Dysplasia patients. *L. Legeai-Mallet, C. Benoist-Lasselin, V. El Ghouzzi, A. Munnich, J. Bonaventure.* Genetique INSERM U393, Hopital Necker, Paris, France.

Achondroplasia (ACH), and Thanatophoric Dysplasia (TD) type I and II, three autosomal dominant chondrodysplasias of increasing severity, have been ascribed to recurrent missense mutations in different domains of the Fibroblast Growth Factor Receptor 3 (FGFR 3). Some of these mutations have been demonstrated to induce constitutive activation of the receptor through a ligand-independent process, but the mechanisms by which FGFR 3 mutations alter bone growth in human are still unclear. Here we show by immunostaining analyses of cartilage sections from ACH and TD I and II patients that overexpression of the mutant receptor was associated to an activation of the Signal transducers and activators of transcription Stat 1 and Stat 5a protein levels in the pre-hypertrophic and hypertrophic chondrocytes of both ACH and TD patients. Expression of the cyclin dependent kinase (cdk) inhibitor p21CIP1, one of the targets for Stat 1/5a was also increased to various extents pending on the severity of the disease. By contrast expression of collagen type X, a specific marker of hypertrophic cells, was dramatically reduced especially in TD I samples, being restricted to the last row of chondrocytes. Evaluation of apoptosis by using the TUNEL assay and quantifying annexin V positive-cells revealed that the number of apoptotic cells in primary cultured chondrocytes derived from the cartilage of control and TD patients raised from < 1% to 5%. We conclude that gain of function mutations in the FGFR 3 gene lead to activation of the Stat pathway by promoting phosphorylation and translocation of Stat 1 and Stat 5a into the nuclei of patient chondrocytes. We speculate that binding of the Stat 1/5a transcription factors to the promoter of the target gene p21CIP1 would induce overexpression of the protein in the cartilage growth plate and premature cell death of abnormally differentiated chondrocytes, thus resulting in growth retardation of long bones.

Two novel retinoic acid-sensitive genes isolated by an induction gene trap approach. *M. Schlicker¹, D.*

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In man and mammals prenatal exposure to the teratogens retinoic acid (RA) and/or retinoids (RX) results in characteristic congenital anomalies including cardiac and craniofacial malformations. Incidence and pattern of the malformations apparently depends on dose and stage of exposure. Yet, the respective molecular mechanisms as well as the genes involved are more or less unknown. RA/RX signalling is mediated by intracellular RA/RX binding proteins and nuclear receptors that activate target genes containing RA-binding elements (RAREs). Based on a retinoic acid-induced gene trap approach (Forrester et al., 1996) we set up a strategy to identify RA/RX downstream genes of mouse and human development that are possible candidate genes to be involved in the generation of congenital malformations. By RACE-PCR of trapped ES clones we identified two novel genes that are expressed in early brain and/or heart. In northern analyses a cDNA fragment of the 1st gene (EScD-1) identifies a brain-specific 6 kb transcript in embryonic midbody (10d p.c.) and adult heart. The 2nd gene (EScD-2) is expressed in fetal brain (2.5 kb) and heart (1.4 kb). By screening of mouse and human Marathon-cDNA libraries we identified about 2 kb (EScD-1) and 1.4 kb (EScD-2) of the respective cDNA sequences including the polyA-tails. The mouse and human sequences of the genes share homologies of 96 % (EScD-1) and 98 % (EScD-2). Searches for human homology revealed that the human gene EScD-2 is represented within human BAC and cosmid sequences mapping to chromosome 16p13.3. Breakpoints within that chromosome segment are known to be associated with cardiac anomalies. Using EScD-1 cDNA primers we isolated a human BAC clone and localized the respective gene on chromosome segment 7q32 by FISH. The detailed analysis of the expression patterns of EScD-1 and EScD-2 by whole mount in situ hybridization and the study of the mouse gene trap phenotypes will give information about the involvement of the two genes in human early development and specific disorders of brain and/or heart. (Supported by DAAD and DFG).

Generation of novel models of congenital anomalies using ENU mutagenesis. *B.J. Herron¹, K.S. Simms¹, H. Peters¹, W. Lu¹, S. Liu¹, M. Justice², D. MacDonald³, D.R. Beier¹.* 1) Genetics Division, Brigham & Women's Hospital/Harvard Medical School, Boston, MA; 2) Baylor University Medical Center, Houston TX; 3) Wichita State University, Wichita, KA.

The generation of models of congenital malformation syndromes can be useful both for the identification of genes important to mammalian development and for understanding the molecular basis of birth defects. We are presently using a systematic approach to screen embryos derived from mice mutagenized with ENU for recessive phenotypes similar to human congenital defects. We use a backcross breeding scheme in which third generation progeny are evaluated at embryonic d18.5. In this manner we identify defects in organogenesis that are consistent with survival *in utero* nearly full term, but which may cause post-natal lethality. Affected progeny from these lines are used directly in genetic mapping utilizing our previously described method of interval haplotype analysis. With this strategy, we are able to localize the mutation to a single linkage group by genotyping 9-10 probands using only 60 markers.

The main component of the mutation screen is a gross assessment of major organs after dissection, followed by a more detailed analysis for anomalies of kidney and brain (by histological examination), and skeleton (by bone stains). Our screening employs a hierarchical strategy in which each familial cohort is analyzed for abnormalities in at least 3 litters. Families for which a significant anomaly is found (about 70%) are further tested by examining 3-6 additional litters for reproducibility of the phenotype. The yield of abnormal phenotypes that appear to segregate in a Mendelian fashion is presently 20% of fully tested families. The spectrum of phenotypes found to date is remarkably varied, and includes developmental disorders of heart, palate, pancreas, kidney, skeleton, muscle and brain. Many of these disorders are similar to human malformation syndromes. Our results demonstrate that a recessive screen can be extremely productive for the purpose of generating abnormal developmental phenotypes and can be readily managed even in a small laboratory.

Role of VAX1 and VAX2 in Holoprosencephaly. J.D. Karkera¹, Y. Du², E. Roessler¹, S. Banfi³, A. Ballabio³, M. Muenke^{1,2}. 1) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia; 3) Telethon Institute of Genetics and Medicine, Milan, Italy.

In order to study the genetic mechanisms of forebrain and eye morphogenesis we have selected two genes from the *Ems/Not* family, VAX1 and VAX2. Recently, a murine VAX1^{-/-} study displayed defective cleavage of the dorsal forebrain into bilateral vesicles resulting in holoprosencephaly (HPE). There also was a failure of choroid fissure fusion leading to optical coloboma. Similarly, overexpression of VAX2 in *Xenopus* leads to enlargement of the optic stalk and aberrant folding of the ventral retina towards the brain. We have determined the intron-exon junction of human VAX1 and VAX2. The alignment study of VAX1 and VAX2 at the protein level suggests that there is a high degree of conservation of the homeodomain region and these proteins are phylogenetically highly homologous. We are in the process of screening for mutations in these genes in HPE patients. The preliminary data obtained thus far: two missense and two silent mutations in VAX2. Both these missense mutations detected are conserved in murine and *Xenopus*. Meanwhile, the normal DNAs will be screened to ascertain whether these changes reflect common polymorphism or novel changes. The DNAs from the respective pedigrees will then be tested for the presence of these particular mutations and further functional studies will be conducted in the early *Xenopus* embryos to evaluate the effect of the mutant VAX2 molecule.

Tracheoesophageal fistula, lung hypoplasia and congenital diaphragmatic hernia: a common pathogenesis of foregut malformation? *C.P. Torfs¹, M.F. van Dooren², D. Tibboel²*. 1) CA Birth Defects Monitoring Program, Emeryville, CA; 2) Sophia Childrens Hospital, Rotterdam, The Netherlands.

Three severe birth defects, congenital diaphragmatic hernia (CDH), tracheoesophageal fistula (TEF) including esophageal atresia and stenosis, and lung hypoplasia (LH) have traditionally been analyzed separately in epidemiological studies. However, recent experimental results in animals have suggested that some common embryological pathways may be shared by the three defects that originate from the budding of the ventral endoderm. Several researchers now postulate that LH may precede or occur concurrently with DH. Experimentally, mutations in several candidate genes such as *Gli2*, *Gli3*, *shh*, *RARab*, *GATA6*, *adrf*, and *HNF-2B* result in the occurrence of one or a combination of these defects. Furthermore, the defects also share environmental risk factors such as vitamin A deficiency and exposure to PHAHs, for example, nitrofen. To evaluate these hypotheses, we studied the prevalence of the combination of these defects in a population of 3,318,966 live and stillbirths in California from 1983 to 1996: There were 493 CDH cases (excluding Morgagni hernia), 893 TEF cases, and 646 LH cases. Of these, 53 (0.2 per 1,000) had TEF + LH and 18 (0.01 per 1,000) had CDH + TEF + LH, a prevalence that is statistically different from the expected ($P < 10^{-6}$ and $P < 10^{-6}$ respectively). Both combinations had, respectively: a male predominance (51% and 56%), a high mortality rate (94% and 85%) and high co-occurrence of multiple associated congenital anomalies, among them: heart defects (76% and 78%), limb reduction defect (34% and 33%), anal atresia, stenosis or imperforate anus (51% and 33%), kidney defects (53% and 6%), genital defects (66% and 50%), CNS defects (41% and 39%), vertebral or rib defects (72% and 57%), digestive tract defects (56% and 41%), and orofacial clefts (11% and 15%). Both groups had 11% of trisomies; all but one were trisomy 18. These observations suggest that mutations in genes crucial to foregut development may result in these combinations of congenital anomalies.

Folate and congenital defects: expression of methylenetetrahydrofolate reductase (*Mthfr*) in early development and effect of folate supplementation in curly-tail (*ct*) mice. P. Tran¹, Z. Chen¹, P. Bagley², J. Selhub², T. Bottiglieri³, R. Rozen¹. 1) Human Genetics, Pediatrics & Biology, McGill University, Montreal, Quebec, Canada; 2) Tufts University, Boston, USA; 3) Baylor University Medical Centre, Dallas, USA.

Folate deficiency is associated with increased risk for neural tube defects (NTD), orofacial clefts (OFC), and congenital heart defects. Periconceptional folate supplementation reduces the occurrence of these anomalies. To investigate the role of folate in early development, we performed two studies: a) we examined the transcriptional expression pattern of *Mthfr* at the time of neural tube closure and b) we assessed the impact of maternal folate supplementation on NTD incidence in the *ct* mouse. MTHFR produces 5-methyltetrahydrofolate, the main circulatory folate derivative. Severe deficiency of MTHFR causes abnormalities of the central nervous system (CNS) and vasculature, while mild deficiency is associated with increased risk for NTD, cardiovascular disease, and possibly for OFC. In situ hybridization of day 8.5, 9.5 and 10.5 C57Bl/6J embryos using an *Mthfr* riboprobe revealed expression in the head, optic eminence, branchial arches, heart and ventral area. From day 8.5 to 10.5, *Mthfr* expression increased notably and progressed rostral-caudally along the ventral region. The expression pattern is compatible with the types of defects seen in our recently-generated knockout mice for *Mthfr* and with the proposed impact of MTHFR on CNS, vascular and orofacial development in humans.

The *ct* mouse is a murine model for NTD, in which 30%-60% of mice are afflicted with tail flexion defects. Data regarding the folate-responsiveness of *ct* mice are inconclusive. Female *ct* mice were administered a folate-supplemented diet at 4 weeks of age, then mated after 8 weeks on diet. Folate levels in maternal livers and in brains of pups reflected the increased folate in the diet. Tail flexion defects were reduced by 25% (p=0.03) in 8 day-old pups. Our studies support the important role of folate in early development.

Characterization of the *Nsdhl* Gene Involved in X-linked, male lethal Chondrodysplasias. X. Wang¹, Q. Ma¹, W. Zhao¹, T. Fox¹, J. Hayes¹, B. Cattana², J. Peters², G.E. Herman¹. 1) Children's Res. Inst., Molecular, Cellular, and Developmental Biology Program, and Depts. of Pediatrics and Pathology, Ohio State U, Columbus, OH; 2) Mouse Genome Centre, Harwell, UK.

Nsdhl is a novel 3 β -hydroxysteroid dehydrogenase that we have recently shown is involved in the removal of C-4 methyl groups in the cholesterol biosynthetic pathway. Mutations in this gene are associated with the X-linked, male lethal mouse mutations bare patches (*Bpa*) and striated (*Str*) and with human CHILD syndrome. *Bpa* mice have a skeletal dysplasia, cataracts, and early psoriatic/ichthyosiform skin changes. The skin changes resolve producing striping of coat. Milder *Str* alleles are normal in size and have a striped coat. Affected human females with CHILD syndrome demonstrate striking unilateral ichthyosiform erythroderma and limb reduction defects. We have now detected missense mutations, V53D and A94T, in conserved amino acids in two additional *Bpa* alleles. The latter mutation is identical to a missense mutation found by Konig et al. (AJMG, 90:339-346, 2000) in 2 unrelated CHILD patients. Thus, differences in the phenotype between *Bpa* mice and CHILD syndrome cannot be completely explained by different types or sites of mutations. The human and mouse NSDHL proteins have a variant form of a Type I ER membrane localization signal, a carboxy terminal RKDK (mouse) and RRVK (human) and one predicted membrane-spanning domain. Using a GFP-tagged NSDHL fusion protein, we have confirmed the expected subcellular localization of the enzyme to the ER membrane. Finally, *Str* male embryos die in midgestation (10.5-2.5 day pc). Placentas from affected male and female *Str* embryos at day 10.5 are smaller than those of normal littermates (p<0.01) and the labyrinthine layer of the placenta appears thinner and disorganized in some affected embryos. In the mouse, the normal paternal X is inactivated in most extraembryonic tissues. Placental abnormalities may contribute to the lethality of the mutation. However, the finding of similar defects in affected male and female placentas suggests that other fetal factors still to be determined must contribute significantly to the eventual male lethality.

Ahch Expression in Murine Embryonic Stem Cells Supports Role Early in Development Prior to Differentiation of Steroidogenic Axis. *R.C. Clipsham¹, Y.-H. Zhang², B.-L. Huang², E.R.B. McCabe^{1,2}.* 1) Molecular Biology Institute; 2) Dept Pediatrics, UCLA Sch Medicine, Los Angeles, CA.

Ahch is the murine homologue of DAX1, a gene that, when mutated, is responsible for adrenal hypoplasia congenita and hypogonadotropic hypogonadism, and, when duplicated, is associated with XY sex reversal. Ahch and DAX1 are known to be expressed in the hypothalamus, pituitary, adrenal cortex and gonads. The purpose of these investigations was to determine whether Ahch was expressed in murine embryonic stem (ES) cells and played a role in development prior to differentiation of the steroidogenic axis. We examined expression by northern blot hybridization and expressed sequence tag (EST) database searches. Ahch was expressed in undifferentiated cultured 129/SvJ ES cells. Preliminary data suggested decreased expression following removal of leukocyte inhibitory factor (LIF), a condition that permitted ES cell differentiation. We are currently examining Ahch expression in cultured ES cells stimulated to differentiate by an alternative mechanism to determine whether or not the observation of decreased Ahch expression is a specific consequence of LIF removal or a general feature of ES cell differentiation. Two other genes, SMRT and NCoR, that code for proteins known to interact with Ahch/DAX1, were also expressed in ES cells. Search of murine EST databases with 20kb of murine genomic sequence containing both Ahch exons identified numerous ESTs from ES cells with 100% matches in the 3' untranslated region. We conclude that Ahch is expressed in undifferentiated ES cells. The expression in these cells of genes coding for proteins known to interact with Ahch/DAX1 is consistent with a functional role for Ahch in undifferentiated ES cells. We speculate that Ahch has a role early in embryogenesis that precedes its later role in the development of the steroidogenic axis.

Midkine is expressed early in rat fetal adrenal development. *P. Dewing*¹, *S. Ching*¹, *Y.-H. Zhang*^{1,2}, *B.-L. Huang*^{1,2}, *E.R.B. McCabe*^{1,2}, *E. Vilain*^{1,2,3}. 1) Dept of Human Genetics, UCLA, Los Angeles, CA; 2) Dept of Pediatrics, UCLA, Los Angeles, CA; 3) Mattel Children's Hospital at UCLA, Los Angeles, CA.

Adrenal gland development is complex and poorly understood at the molecular level. Only a subset of patients with adrenal hypoplasia congenita (AHC) carry mutations in DAX1, a member of the nuclear hormone receptor superfamily. Therefore we set out to identify other candidate genes responsible for AHC by characterizing genes involved in fetal adrenal development. To identify these genes, we studied differential gene expression in embryonic rat adrenals comparing 13-14 with 15 dpc, since these two time points encompassed a period of major morphological change. Fetal rat adrenals were dissected, cDNAs prepared and suppressive subtractive hybridization performed. We isolated 192 clones of putatively differentially expressed clones. Approximately 400bp of each of the clones were sequenced and the most relevant putative developmental genes were examined.

A member of the PTN/MK (pleiotrophin/midkine) heparin-binding protein family was selected for initial study. This is a family of extracellular heparin-binding proteins involved in regulation of growth and differentiation. Midkine(MK) was originally cloned in search for genes transcribed during retinoic acid-induced differentiation. Its expression is ubiquitous at 7-9 embryonic days in the mandible/maxilla, lungs, caudal sclerotomes, limbs and brain ventricles, but limited to the kidney at day 15.

We obtained full length transcript by 5' Rapid Amplification of cDNA Ends (RACE) and performed northern analysis on rat adrenal RNA from fetuses at 13, 14, 15, 17, 19 dpc and newborns. Results from those analyses demonstrated the highest MK expression at days 13 and 14 followed by a moderate decrease of expression during the fetal stages thereafter. At the newborn stage, however, MK expression is nearly undetectable. Our results indicate that MK has a very specific pattern of fetal expression in the adrenals. We conclude that MK is involved early in fetal development of the rat adrenal. Therefore, MK is a candidate genes for AHC not due to DAX1 mutations.

Mis-expression of human cytochrome P450 (CYP2D6*17) in the *Drosophila* wing results in wing patterning defects. *L.T. Reiter*¹, *B. Biehs*¹, *D. Ang*¹, *M. Oscarson*², *E. Bier*¹. 1) UCSD, La Jolla, CA; 2) Karolinska Institutet, Stockholm, Sweden.

Cytochrome P450s (C-P450) are a group of mixed function monooxygenases that metabolize drugs and toxins in both humans and *Drosophila*. It is estimated that human C-P450 *CYP2D6* is responsible for the metabolism of over 30 clinically relevant drugs like debrisoquine, commonly used for the treatment of hypertension. C-P450s may also be involved in the synthesis of small signal molecules during development as suggested by the finding that mutations in human *CYP1B1* lead to primary congenital glaucoma (PCG). We have begun analyzing the roles of *CYP2D6* and *CYP1B1* during development. This analysis is twofold: mis-expression of wild-type and mutant forms of these proteins in the *Drosophila* wing using the GAL4-UAS system and mutagenesis of the *Drosophila* gene encoding the homologue of *CYP2D6*, *CYP1B1*, *CYP21* and *CYP17*, known as *cyp18*. cDNAs encoding the wild-type human *CYP2D6* gene, a putatively non-heme binding mutant form *CYP2D6-T107A* (*CYP2D6*17*), and wild-type versions of human *CYP1B1* and *Drosophila cyp18* were placed under the control of the yeast upstream activator sequence (UAS) and introduced into the fly genome. Mis-expression of wild-type UAS-*CYP2D6* driven by the yeast GAL4 transactivator results in a few ectopic wing veins and reduction of endogenous *cyp18* expression in the developing wing disk. Mis-expression of *CYP2D6*17* induces a significant amount of ectopic vein formation between L2 and L3 and squares off the wing margin. Misexpression of *CYP2D6*17* also alters endogenous *Drosophila cyp18* expression within the wing pouch. These results suggest that the human C-P450s are able to disrupt the normal *cyp18* expression pattern by binding to and/or modifying the signal molecules synthesized by *cyp18*. Attempts to disrupt the *cyp18* gene via a PCR based P-element mutagenesis scheme are underway. We expect that these studies in *Drosophila* will lead to a mechanistic understanding of the process by which human C-P450 mutations result in disorders such as PCG (*CYP1B1*) and adrenal hyperplasia (*CYP17* and *CYP21*).

Notch1, Notch2, Notch3 and Notch4 are expressed in the developing mouse liver. *R.J Oakey¹, N.B. Spinner¹, D.A. Piccoli², K.M. Loomes^{1,2}.* 1) Division of Human Genetics, CHOP, Philadelphia, PA; 2) Division of Gastroenterology, CHOP, Philadelphia, PA.

Introduction: Alagille syndrome (AGS) is an autosomal dominant disorder characterized by paucity of the intrahepatic bile ducts in combination with developmental abnormalities of the heart, eye, skeleton, face and kidney. Mutations in human Jagged1, a ligand in the Notch intercellular signaling pathway, have been found to cause AGS. We have demonstrated previously that Jagged1 expression in the developing mouse and human liver co-localizes with the primitive bile duct cells or ductal plate. In order to determine which of the Notch receptors interacts with Jagged1 in the liver, we have assayed expression of the Notch genes during development. Methods: RNA and serial sections were generated from staged mouse embryos and developing mouse livers. Expression of Notch1, Notch2, Notch3 and Notch4 was assayed by RT-PCR and in situ hybridization. Results: Notch1,2,3 and 4 are expressed at all stages assayed in the mouse from 7.5 days post coitum (dpc) to 14.5 dpc. Notch1,2,3 and 4 are expressed in embryonic liver at 13.5 dpc, the stage at which bile duct development begins. All four mouse Notch genes are also expressed in 10 day postnatal liver, 3 week old liver and adult liver. In situ hybridization studies show a diffuse pattern of expression in the liver at 12.5 dpc, 14.5 dpc and newborn liver for Notch1,2 and 4. Mouse Notch3 is expressed in the newborn liver in a ring-like pattern surrounding the vascular structures, similar to the expression pattern of Jagged1 at the same stage. Conclusions: All four Notch genes are expressed in the mouse liver from mid-gestation through adulthood. One or all of these Notch receptors could interact with the ligand Jagged1 during liver and bile duct development. Mouse Notch3 mRNA is spatially localized with Jagged1 in the neonatal liver as shown by in situ hybridization. This specific expression pattern may imply a role for mouse Notch3 in signaling during bile duct development. Functional studies are necessary to define further the specific ligand-receptor relationships involved in bile duct development.

Activin signaling and gene dosage: evidence for both endocrine and autocrine/paracrine effects *in vivo*. C.W.

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Activins A and B (dimers of bA or bB subunits) are TGF- β superfamily members that play critical roles in reproduction and development. Recently, we have shown that activin bB is sufficient to rescue the phenotypes of activin bA null mutant mice, by creating knock-in mice that produce activin bB at sites where activin bA is normally expressed. However, novel phenotypes were observed, suggesting that despite overlapping functions *in vivo*, the activins are not entirely functionally interchangeable. To further assess the influences of gene dosage and endocrine versus autocrine/paracrine effects, we have produced mice that express activin bB from the activin bA locus, but not from the wild type activin bB locus. Similar to activin bB knockout mice, double homozygous mutant mice [actbA^{BK/BK}; actbB^{-/-}] have eyelid closure defects. Thus, despite its widespread expression during embryonic development, including the developing orbit, activin bB produced in an activin bA expression pattern cannot rescue the eyelid closure defects of the activin bB knockout mouse. In contrast to both actbB^{-/-} knockout mice and homozygous activin bB knock-in (BK/BK) mice, which are viable and fertile, the double homozygous mutant mice generally do not live beyond the first week. Those that survive have profound somatic and gonadal growth deficiencies, delayed hair growth, and enlargement of the external genitalia. The phenotypic characteristics of the double mutant mice support the hypothesis that critical developmental functions, including survival beyond the neonatal period and somatic growth, are supported by both activins A and B in a dose-dependent fashion, although activin A plays a more important role in this context. In contrast, specific processes such as eyelid fusion are exquisitely sensitive to correct spatio-temporal activin expression, consistent with autocrine/paracrine roles for the activins. We will discuss the mechanisms by which activins influence the growth and fertility phenotypes of the knock-in mice.

***Chx10-1*, a rapidly evolving murine homologue of the paired-like homeodomain transcription factor *Chx10*.** R.L. Chow¹, B. Snow¹, J. Novak¹, J. Looser¹, C. Freund¹, B. Chang², M. Davisson², B. Taylor², L. Ploder¹, R.R. McInnes¹. 1) Program in Developmental Biology, Research Institute, Hospital for Sick Children, Toronto, ON, Canada; 2) The Jackson Laboratory, Bar Harbor, ME.

The homeodomain (HD) transcription factor CHX10 is essential for eye development. *Chx10* loss-of-function mutations cause the ocular retardation mutation in mice, and similar *CHX10* alleles in humans lead to microphthalmia and blindness. To identify *Chx10* homologues, we conducted a low-stringency cDNA library screen and isolated a murine homologue, *Chx10-1*. The *Chx10-1* predicted protein is closely related to CHX10 and VSX1 (a *Chx10* homologue in human) in the conserved HD/CVC domains (86% and 93% identical, respectively), but outside these domains, the identity is < 63%. *Chx10-1* and *VSX1*, appear to be orthologues, however, since (i) they map to regions of conserved synteny in mouse and human (*Chx10-1* to chr. 2, close to *coloboma* and *blind sterile*, *VSX1* to chr. 20p11-q11), and (ii) we have not identified a *VSX1* orthologue in mouse nor a *Chx10-1* orthologue in human, despite careful screening. To start defining the function of *Chx10-1*, we examined its expression. RNA blots of adult mouse tissues showed a 3.4 kb *Chx10-1* mRNA in retina but not in 7 other tissues. Both *Chx10* and *Chx10-1* are expressed in retinal progenitor cells at E15.5 but, whereas differentiating ganglion cells upregulate *Chx10-1*, their formation is associated with extinction of *Chx10*. *Chx10-1* is also expressed in the lens epithelium, developing Harderian glands and eyelids. In the mature retina, *Chx10-1* transcripts are restricted to the inner nuclear (INL) and ganglion cell layers; outside the eye expression is most prominent in the developing nasal epithelium. We conclude that (i) *Chx10-1* is a member of the CVC domain group of paired class homeodomain proteins, (ii) the overlapping expression of *Chx10* and *Chx10-1* in the INL may specify the cells of this layer, (iii) *Chx10-1* is a strong candidate gene for *coloboma* and *blind sterile*, and mutations in *Chx10-1* may cause defects in the neural retina and optic nerve, (iv) *Chx10-1* and human *VSX1* may be rapidly diverging orthologues partly responsible for eye speciation in mouse and man.

Genetic interactions of *Pitx2* with *Lhx3* and *Lhx4* in pituitary development. *P. Eswara*¹, *H. Suh*², *A.L. Radak*¹, *P.J. Gage*¹, *S.A. Camper*^{1,2,3}. 1) Dept Human Genetics; 2) Neuroscience Program; 3) Dept of Internal Medicine, University of Michigan, Ann Arbor, MI.

Mutations in the homeodomain transcription factor PITX2 lead to Rieger syndrome (RGS), a dominant haploinsufficiency disorder with early onset glaucoma, iris hypoplasia, tooth defects, and umbilical hernia (1). Heart defects and pituitary-based growth insufficiency are occasionally found. Mice heterozygous for a *Pitx2* null allele exhibit a low frequency of RGS features (2). The variable phenotype in both humans and mice suggests the possibility of genetic background effects. To assess this, we transferred the *Pitx2* null allele to four inbred strains. At generation N3, no obvious tooth or growth defects were observed, and analysis of the eyes is in progress. We tested interaction of *Pitx2* with *Lhx3* and *Lhx4*, two LIM homeobox genes that are required for pituitary organogenesis. Both *Lhx3* and *Pitx2* mutants have arrested pituitary development after initial formation of Rathke's pouch (2, 3). *Lhx4* mutants have a milder pituitary phenotype (4). The functions of *Lhx3* and *Lhx4* overlap and the dosage of each gene influences pituitary organogenesis. To determine whether *Pitx2* function overlaps with that of either of these genes we bred *Lhx3*^{+/-} and *Lhx4*^{+/-} mice to *Pitx2*^{+/-} mice. The F1 double heterozygotes from both the crosses were viable, fertile and obtained in Mendelian ratios. Preliminary analysis of embryos from *Lhx3*^{+/-}, *Pitx2*^{+/-} intercross and *Lhx4*^{+/-}, *Pitx2*^{+/-} intercross suggested that the *Lhx3*^{-/-}, *Pitx2*^{-/-} and *Lhx4*^{-/-}, *Pitx2*^{-/-} embryos are more severely affected than either of the single mutants. Because mutations in *LHX3* cause combined pituitary hormone deficiency (CPHD) (5), and both *Lhx4* and *Pitx2* overlap functionally with *Lhx3*, interactions between these genes could contribute to CPHD.

1.Nat Gen 14, 392-399, 1996.

2.Development 126, 4643, 1999.

3.Science 272, 1004, 1996.

4.Science 278, 1809. 1997.

5.Nat Gen 25,182, 2000.

Autosomal dominant polycystic kidney disease: More than a renal disease. *J. Horst¹, P. Pennekamp¹, W. Bloch³, B. Skryabin², K. Addicks³, B. Dworniczak¹*. 1) Institute of Human Genetics, University of Muenster, Germany; 2) Institute of Experimental Pathology, University of Muenster, Germany; 3) Institute I of Anatomy, University of Cologne, Germany.

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most frequently inherited monogenic diseases and affects up to 1:1000 individuals worldwide. It is characterized by progressive development and enlargement of fluid filled cysts in the kidneys that frequently result in chronic and end-stage renal failure. Approximately 8-12% of all patients requiring haemodialysis suffer from this disease. However it is not only restricted to the kidneys but can also manifest as a multisystem disorder, with symptoms including arterial hypertension, hepatic cysts, cardiovascular valve abnormalities, pancreatic cysts, cerebral aneurysms and colonic diverticuli. To analyse the function of the ADPKD genes in more detail we at first characterised the mouse *Pkd2* gene, analysed its expression pattern in comparison to *Pkd1* during development and secondly created a mouse model for ADPKD2 by homologous recombination. Using conventional gene targeting technologie we replaced exon one of the *Pkd2* gene by the *LacZ* gene. Beside some single episodes like sudden death due to most probably bleeding into the thoracic region of pregnant heterozygous mice and cysts in the testicle region of one male chimaera heterozygous *Pkd2* knockout mice do not show any phenotype so far, whereas the homozygous *Pkd2* mutant mice are not viable and die pre- or perinatally between day 13.5 and birth with multiple defects. These defects include the previously described bilateral cystic kidneys, cysts in the pancreas, hypoplasia of the lungs, whole body oedema with bleedings and heart defects. In addition semithin sections and electron microscopy revealed a massive involvement of microvessels during the pathogenesis of ADPKD with defects in the endothelium. In contrast to other groups we were able to detect defects of the liver and biliary ducts like necrosis and morphological changes in the biliary epithelium. Confirmation of previously published results and additional information will be presented.

Global analysis of gene expression patterns during differentiation of embryonic stem cells. *S.A. Jaradat, T. Tanaka, L. O'Neill, K. Boheler, M.S.H Ko.* Lab Genetics, NIH/NIA, Baltimore, MD.

Mouse embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of the pre-implantation mouse embryo. Pluripotency of ES cells can be sustained in vitro in the presence of leukemia inhibitory factor (LIF). The mechanisms underlying the maintenance of ES cell differentiation when LIF is withdrawn remain elusive. To further our understanding of the pathway(s) controlling ES cell pluripotency, we examined gene expression patterns on a genomic scale, using a cDNA microarray. For this purpose, we constructed a microarray containing 15,000 mouse cDNAs, 50% of which were derived from early developmental stages. We compared gene expression in ES cells in the presence of LIF with gene expression at various time points post LIF withdrawal (4, 8, 18, 24, 36 hours and embryoid bodies). A hierarchical clustering program grouped the 15,000 genes on the basis of their expression profiles over time. A distinct cluster contained genes that were sharply down regulated 4 hours post LIF removal. This category contains well characterized genes, such as Stat1, Oct-4, Rex-1 and SOX-2, that are expected to be down regulated by LIF withdrawal. A second cluster consisted of genes upregulated during differentiation included genes such as e-Hand, which is important in heart development. Two general clusters are emphasized in this abstract, but there were also further subdivisions of genes whose expression, showed sharp upregulation or downregulation during differentiation. Novel gene clusters observed in this study, provide a starting point to assess regulatory factors and mechanisms leading in early differentiation. *stat1oct-4Rex-1Sox-2e-Hand*.

Developmental role for ataxin-2 and the fox-1 homolog A2BP1. *T.-R. Kiehl¹, A. Nechiporuk², D.J. Luthringer³, S.-M. Pulst¹.* 1) Dept. of Neurology, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Dept. of Human Genetics, Howard Hughes Medical Institute, University of Utah, Salt Lake City, UT; 3) Dept. of Pathology, Cedars-Sinai Medical Center, Los Angeles, CA.

Ataxin-2, the gene product of the human spinocerebellar ataxia type 2 (SCA2) gene, is a protein of unknown function. It interacts with ataxin-2-binding-protein 1 (A2BP1), a member of a novel family of putative RNA binding proteins. Both are highly conserved among species, with orthologs in mouse, fly and worm. A2BP1 has 29.8 % aa identity to its worm ortholog, fox-1. Fox-1 is known to be a numerator element, but its role in development was unknown. Using RNA interference, we show that both proteins play a role in early embryonic development in the worm. There was a marked reduction in the number of embryos, with an average of 11 offspring per worm observed in the atx-2(RNAi) group, 94 in the fox-1(RNAi) group and 250 in the control. We generated an ataxin-2 null mouse. Animals are viable with no gross anatomic defects. There is no reduction in fertility or segregation distortion with an average of 6.8 mice (SD=1.32) per litter in the wild type and 6.25 (SD=1.26) in the homozygous knockout group. Deletions or loss of function mutations involving either gene are not known in humans. We stained a human ovary specimen containing a large number of primary follicles and oocytes with antibodies to ataxin-2 and A2BP1. There was strong overall staining for ataxin-2 both in follicles and in the surrounding stroma. For A2BP1, we observed highly specific staining in the oocyte and in the zona pellucida. Staining was confined to the cytoplasm of the oocyte whereas the nucleus was spared. This is consistent with observations in neuronal and non-neuronal cell types showing that both ataxin-2 and A2BP1 colocalize with the trans-golgi network. It is also compatible with previous findings implicating the worm orthologs atx-2 and fox-1 in embryonic development. Since both have RNA-binding properties, a function in RNA distribution or processing is plausible. Further studies will elucidate the role of these proteins in mammalian embryonic development and assess possible implications for human reproductive medicine.

***Lmx1b* regulates type IV collagen gene expression and underlies renal dysplasia in nail patella syndrome. R.**

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Nail patella syndrome (NPS) is a dominantly inherited skeletal dysplasia associated with nephropathy and is caused by mutations in the LIM homeodomain transcription factor *LMX1B*. Patients with NPS have nephropathy associated with albuminuria, and ultrastructural abnormalities of glomerular basement membranes (GBM), and of podocyte foot processes. Among the components of the glomerular filtration barrier is a specialized basement membrane (BM) that, in humans and rodents, undergoes a developmental switch of extracellular matrix expression. This switch occurs from the expression of the $\alpha 1$ and $\alpha 2$ subunits of type IV collagen together with the b1 form of laminin during the fetal life to the expression of the $\alpha 3$, $\alpha 4$ and $\alpha 5$ subunits of type IV collagen and the S-laminin isoform, in late fetal and adult life. We hypothesized that *Lmx1b* may regulate matrix expression during GBM development. We observed reduced expression and absence of mRNA for *Col4a3* and *Col4a4*, respectively, in *Lmx1b*^{-/-} newborn mouse kidneys compared to wild-type littermates. The glomeruli as well as the cells secreting these extracellular molecules are preserved, as shown by appropriate expression of *sFrp2* and *Col4a1*. Moreover, LMX1B is able to bind to a putative enhancer-like sequence for *COL4A3* and *COL4A4* that we identified at the 3' end of the first intron of *COL4A4*. These genes are arranged in a head to head fashion and are transcribed in opposite directions in coordinate fashion. Finally, LMX1B can upregulate the reporter activity of a construct containing this enhancer-like element positioned upstream of a minimal promoter. These data together, identify *COL4A4* as the first downstream target of *LMX1B* described to date and suggest that a disruption of GBM morphogenesis underlies the renal dysplasia observed in NPS. Moreover, the abnormal podocyte architecture in NPS patients and *Lmx1b*^{-/-} mice may point to more global dysregulation of podocyte development.

FGF-10 Initiates Ocular Gland Morphogenesis. *P.A. Overbeek, V. Govindarajan.* Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX.

Purpose: FGF-10, a member of the fibroblast growth factor family, is thought to mediate epithelial-mesenchymal interactions in different organ systems. The purpose of this study was to test whether FGF-10 regulates normal ocular development. **Methods:** An α A-crystallin promoter was used to obtain lens-specific expression of FGF-10 in transgenic mice. Expression of FGF-10 in transgenic and wild type mice was examined by in situ hybridizations using ³⁵S-labelled riboprobes. Lacrimal gland development was studied using transgenic mice that express lacZ driven by a 5 kb Pax-6 promoter. Ocular morphogenesis in wild type, transgenic, and FGF-10 knock-out mice was analyzed by standard histological techniques. **Results:** Lens-specific expression of FGF-10 induced proliferation of the corneal epithelial cells followed by inward growth and invasion of the corneal stroma. The corneal epithelium subsequently differentiated into gland-like structures that resemble the lacrimal and Harderian glands. Endogenous expression of FGF-10 was seen in the mesenchymal cells surrounding both the lacrimal and Harderian gland primordia during initiation of glandular development. In addition, lacrimal and Harderian glands were not induced in FGF-10 null fetuses. **Conclusions:** FGF-10 is both necessary and sufficient for induction of ocular gland differentiation. FGF-10 expression in the perioptic mesenchyme acts to alter the fate of the adjacent epithelial cells in the conjunctiva and to initiate glandular development. Supported by NIH grants EY10448 and EY10803.

Localization of angiotensinogen-expressing cells in the fetal kidney: Clues into the role of Angiotensin in Renal

Development. *M.C. Prieto*^{1,2}, *S. Dipp*², *S. Meleg-Smith*³, *S.S. El-Dahr*². 1) Physiology Department, Tulane University School of Medicine, New Orleans, LA; 2) Pediatrics Department, Tulane University Medical Center, New Orleans, LA; 3) Pathology Department, Tulane University School of Medicine, New Orleans, LA.

The epithelial anlagen in the developing kidney undergo a process of centrifugal maturation characterized by intense proliferation and segmental patterning followed by structural and functional differentiation. The renin-angiotensin system is essential for normal renal growth and functional maturation. However, the precise developmental stage(s) of nephrogenesis that are regulated by angiotensin II are not completely known. To gain further insights into the developmental role of intrarenal angiotensin II, we examined the localization of its precursor, angiotensinogen (Ao), in the rat fetal kidney utilizing immunohistochemical techniques. Distinct expression of Ao in the tubular epithelium was not apparent until segmental nephron patterning was established on E16. At this stage, Ao was expressed at low levels in ureteric bud ampullae, but not in the metanephric-derived epithelial anlagen. A marked induction in the abundance of Ao coincided with terminal epithelial differentiation. On E17, Ao was expressed in the mesangium of cup-shaped glomeruli and the proximal tubular brush border. The classical adult localization pattern of Ao in well differentiated proximal tubules was clearly established by E18-E19. Immunostaining for the DNA replication factor, proliferating cell nuclear antigen (PCNA), revealed that the distribution of cyclin cells was confined to the metanephric mesenchyme, the branching ureteric buds, and the epithelial anlagen. In contrast, Ao-containing tubular epithelial cells were PCNA-negative. These results demonstrate that expression of Ao correlates spatiotemporally with terminal epithelial differentiation. This pattern of developmental expression infers that intrarenally produced angiotensin II regulates differentiated tubular functions and may be important for the maintenance of the differentiated tubular epithelial phenotype.

Perturbation of the developing chick embryo using antisense to fibrillin-1. *S. Plaza*¹, *G. Zhou*¹, *S. Belleh*¹, *S.V. Vinogradov*², *A.V. Kabanov*², *M. Godfrey*¹. 1) Ctr for Human Molecular Genetics; 2) Dept Pharmaceutical Science, Univ Nebraska Med Center, Omaha, NE.

Mutations in fibrillin-1 (FBN1) a microfibrillar glycoprotein with a ubiquitous distribution, are known to cause the Marfan syndrome (MFS). Given the pathophysiology of MFS, it has been hypothesized that fibrillin-1 plays primarily a "load bearing" function in the extracellular matrix. However, it has been shown that fibrillin-1 is expressed as early as the primitive streak in the developing chick embryo. Since "load bearing" is not a critical function at that stage of development, we used an antisense approach to target fibrillin-1 during early chick development. Our initial studies were focused on getting antisense oligonucleotides into the embryo. Using fluorescence tagged oligonucleotides, we observed a greater accumulation of oligonucleotide in the embryo if the oligonucleotides were bound to cationic block copolymers of polyethyleneglycol and polyethylenimine.

Block copolymer bound phosphorothioate antisense oligonucleotides were inoculated into eggs four hours post fertilization. Additional inoculations of oligonucleotides or controls were at 24, 48, 72, 96, and 120 hours of fertilization. 50ml of a 10mM concentration of oligonucleotide was used per inoculation. Control eggs were inoculated with equivalent volumes of PBS, copolymer alone, antisense oligonucleotides without copolymer, and sense oligonucleotide with copolymer. At 6 days, normal chick embryos should be at Hamburger and Hamilton stage 29. All control embryos were at normal stages despite inoculation with various compounds. The embryos inoculated with antisense to fibrillin-1 without copolymer were also normal. Embryos inoculated with antisense to fibrillin-1 with the block copolymer showed a range of abnormalities ranging from delay in development to gross morphological abnormalities. A similar trend is seen after a three day incubation. These studies point to the critical role fibrillin-1 must play in early embryogenesis and the greater efficiency and efficacy of block copolymer bound antisense oligonucleotides.

High throughput screening of the genes related with postnatal development of rat cerebellum. *Y.H Kang, H. Kim, I.J. Rhyu, C.M. Kim, Y.S. Suh, S.H. Park.* Institute of Human Genetics, Dept. of Anatomy, Korea Univ. Coll of Medicine, Seoul, Korea.

To clone the genes related with postnatal development of cerebellum, we screened developmentally differentially expressed genes from rat brain with ordered differential display PCR (ODD-PCR) and cDNA microarray. The expression patterns of cloned genes in the developing rat cerebellum were investigated with *in situ* hybridization histochemistry. In this experiment, we screened developmentally expressed genes from 12 cDNA pools (E12, E14, E16, E18, E20, P0, P3, P7, P12, P18, P25, and adult rat brain) with ODD-PCR. Among 20000 developmentally differentially expressed cDNA bands, 3000 cDNAs were subcloned into T easy vector, sequenced, and searched. 1500 clones were novel genes showing very low sequence homology with registered genes, 820 clones were homologous with registered genes, and 680 clones were almost identical with registered genes. The expression patterns of these genes in the postnatal cerebella were investigated by cDNA microarray. While the majority of transcripts were expressed at the similar level from postnatal day 3 (P3) cerebellum to adult cerebellum, more than 100 mRNAs were modulated during postnatal development of cerebellum. *In situ* hybridization histochemistry was performed to see the expression of screened genes in the tissue sections of developing rat cerebellum. More than 60 genes encoding doublecortin, vimentin, VAMP-1, glutamate receptor subunit, myelin basic protein, and etc showed specific expressional changes. A possible roles of these known genes in the postnatal development of cerebellum will be discussed.

***Zic3*-deficient mice manifest defects in left-right axis development.** *S.M. Purandare*¹, *M. Gebbia*¹, *M.T. Bassi*¹, *K.M. Kwan*², *H. Vogel*¹, *R. Behringer*², *B. Casey*¹. 1) Dept Pathology, Baylor Col Medicine, Houston, TX; 2) Dept Molecular Genetics, Univ Texas MD Anderson Cancer Center, Houston, TX.

A cascade of asymmetric gene expression initiated at the vertebrate node controls the positioning of the heart and other organs along the left-right (LR) axis. Previously we found that some cases of LR anomalies are associated with mutations in *zinc finger in cerebellum 3* (*Zic3*), a gene on the human X chromosome. A targeted deletion of murine *Zic3* was engineered in order to begin elucidating the role of this transcription factor in mammalian LR specification. All *Zic3*-null mice that survive intrauterine development have a kinked tail. Approximately 25% manifest, alone or in combination, malformations of the central nervous system (CNS), the axial skeleton, and the left-right (LR) axis. Similar to the human phenotype, LR anomalies were of random, indeterminate sidedness (*situs ambiguus*). Malformations of the CNS (exencephaly accompanied by agenesis of the cerebellum) and those of the axial skeleton (unilateral homeotic transformations) appeared independently of LR-axis anomalies and with equal frequency. Symmetric *nodal* expression at the node in *Zic3*-deficient mice begins appropriately but disappears rather than becoming left-dominant as in the wild type. Subsequent expression of *nodal* and the downstream gene *Pitx2* in the lateral plate mesoderm is randomized rather than consistently left-sided. These results suggest a role for *Zic3* in murine LR development by helping to maintain *nodal* expression at the node.

Limb and CNS malformations in *Lhx2*^{-/-}:*Lhx9*^{-/-} mutant embryos. C.A. Wassif¹, Y. Zhao², S. Mackem³, H. Westphal², F.D. Porter¹. 1) HBD/NICHD/NIH, Bethesda, MD; 2) LMGD/NICHD/NIH, Bethesda, MD; 3) NCI/NIH, Bethesda, MD.

Lhx2 and *Lhx9* are two closely related LIM/Homeobox genes with overlapping, but distinct developmental expression patterns. In order to determine the developmental functions of these genes, we disrupted both genes in ES cells. *Lhx2* is necessary for forebrain and eye development, as well as definitive erythropoiesis. *Lhx9* is necessary for gonad formation. Both *Lhx2* and *Lhx9* are co-expressed in the progress zone of the developing limb, and *Apterous*, the drosophila homolog of *Lhx2* and *Lhx9*, is necessary for normal wing development. Neither *Lhx2*^{-/-} nor *Lhx9*^{-/-} embryos have any limb malformations. *Lhx2* and *Lhx9* are also coordinately expressed in the developing midbrain. Hence we proposed that the combined mutant embryos would have a limb defect, and additional CNS defects beyond those seen in the *Lhx2*^{-/-} embryos. In the midbrain of *Lhx2*^{-/-}:*Lhx9*^{-/-} embryos we find that the diencephalon is hypoplastic with dilatation of the third ventricle. The limbs of *Lhx2*^{+/-}:*Lhx9*^{+/-}, *Lhx2*^{+/-}:*Lhx9*^{-/-}, and *Lhx2*^{-/-}:*Lhx9*^{+/-} mice develop normally. However, both the fore and hind limbs of the *Lhx2*^{-/-}:*Lhx9*^{-/-} mice fail to develop properly. Although the limb buds of the mutant embryos are smaller, the AER appears normal. Analysis of alizarin and alcian blue stained embryos demonstrated that the mutant limbs have a progressive loss of normal structures in the proximal to distal axis. The humerus and the femur are relatively normal. The bones of the zygopodium are present, but markedly shortened and malformed. The malformed limbs then terminate in two shortened phalangeal elements. This phenotype is consistent in eight double mutant mice evaluated to date. *In situ* analysis of the expression patterns of genes involved in limb development and BrDU analysis is in progress. Based on our prior knowledge that both *Lhx2* and *Lhx9* are important for cellular proliferation in other developmental fields, we propose that these two genes are functionally redundant but necessary for the proliferation of cells in the progress zone of the developing limb. Lack of this cellular proliferation leads to the progressive loss of limb structures in the proximal to distal axis.

Gene expression profiles from early stages of human craniofacial development. *L.Q Zhang¹, T. Attié-Bitach², Y. Korshunova³, J. Cai¹, D. Messina³, J. Augé², M. Vekemans², E.W Jabs¹, M. Lovett³.* 1) Johns Hopkins University, Baltimore, MD; 2) Hospital Necker Enfants Malades, Paris, France; 3) Washington University School of Medicine, St. Louis, MO.

The Craniofacial and Oral Gene Expression Network (COGENE) is employing micro-cDNA methods, serial analysis of gene expression (SAGE), Affymetrix chip analyses and subtractive cDNA techniques to analyze gene expression in early human development and especially in microdissected, embryonic craniofacial structures. We have generated cDNA libraries from a 26-day conceptus (4mm crown rump), from fourth week rhombomeres (numbers 1 through 4) and from third week 1st pharyngeal arch as the first steps in this project. DNA sequencing of several hundred clones from each of these libraries indicates that ~20% of cDNAs encode proteins involved in protein synthesis or cell division, reflecting the enormous growth and proliferation that is occurring during these stages. Interestingly, these sequences also include multiple copies of cDNAs encoding putative tumor suppressors and proto-oncogene products such as DOC-1, JUND, QM and AF1q as well as transcription factors such as PLZF and ILF3. These analyses were validated and extended by a modified micro-SAGE method. Sequencing of 50,864 SAGE tags from the 26-day conceptus, yielded 20,133 different 10-bp tags, 12,193 of which matched known genes or ESTs. The remaining 7940 (39%) tags presumably represent novel genes. 14,908 tags were found once, while 5,225 tags occurred multiple times, ranging from 2 to 620 times. In agreement with the cDNA analyses, the most highly expressed class of transcripts (18% of all tags) were from genes encoding ribosomal proteins. Included among the 20 most highly expressed transcripts were 1 glutamate receptor gene, 2 cystatin C genes, 2 CD74 antigen genes, 1 translation elongation factor, 1 epsilon 1 hemoglobin gene, 1 ATPase, 2 ESTs and 1 non-matching tag. The unique libraries and the data derived in this project will provide new insights into which specific genes and pathways are being expressed during human craniofacial and oral development and are accessible at <http://hg.wustl.edu/lovett/projects/cogene>.

Mutations of *PVRL1*, encoding a cell-cell adhesion molecule/Herpesvirus receptor, in cleft lip/palate-ectodermal dysplasia. R.A. Spritz¹, K. Suzuki¹, D. Hu², T. Bustos³, J. Zlotogora⁴, A. Richieri-Costa⁵, J.A. Helms². 1) Univ. Colorado Health Sci Ctr, Denver, CO; 2) Univ California San Francisco, CA; 3) Univ Central de Venezuela, Caracas; 4) Hebrew Univ, Jerusalem, Israel; 5) HRAC-USP, Bauru, Brasil.

Cleft lip \pm cleft palate (CL/P), is among the most common birth defects, occurring in 0.4 to 2.0 per 1000 liveborns. About 70 percent of CL/P is non-syndromic, but CL/P also occurs in many single-gene syndromes. We have positionally cloned the gene for CLPED1, an autosomal recessive CL/P-ectodermal dysplasia (ED) syndrome (ED4; Margarita Island ED; Zlotogora-Ogur syndrome), characterized by CL/P, hidrotic ED, developmental defects of the hands, and in some cases, mental retardation. We identify the CLPED1 locus as *PVRL1*, encoding an immunoglobulin-related transmembrane cell-cell adhesion molecule, part of the NAP cell adhesion system. In addition, *PVRL1* is the principal receptor for cellular infection by HSV1 and HSV2. We identified 3 independent null-mutant alleles in patients with different clinical forms of CLPED1, not previously realized to be allelic. A W185X nonsense mutation is ubiquitous on Margarita Island, at a high heterozygote frequency (1/26), possibly the result of resistance to infection by HSV1 and HSV2. In mouse embryos, we find that *PVRL1* is expressed in day e15-15.5, primarily in medial edge epithelium of the palatal shelves, the ectodermal component of tooth buds, olfactory epithelium, and skin surface epithelium, consistent with the phenotype of CLPED1. *PVRL1* may also provide clues to other types of CL/P. The high frequency of CLPED1 carriers on Margarita Island may contribute to the high frequency of non-syndromic CL/P on the island. Furthermore, two close *PVRL1* paralogues, *PVR* and *PVRL2*, likewise encode Ig-class cell adhesion molecules that serve as viral receptors and are both located in chromosome segment 19q13.2-q13.3 nearby *BCL3*, the position of OFC3, one of several non-syndromic CL/P susceptibility loci mapped in humans. We are currently studying genetic variation of *PVRL1*, *PVRL2*, and *PVR* in patients with non-syndromic CL/P, including families exhibiting apparent linkage to OFC3.

Perinatal Development of Endothelial Nitric Oxide Synthase Deficient Mice. *L.A. Hefler¹, C.A. Reyes², B.B.*

Leibman¹, W.E. O'Brien³, A.R. Gregg^{1,3}. 1) Obstetrics & Gynecology, Baylor College of Medicine, Houston, TX; 2) Department of Pathology, Baylor College of Medicine, Houston, TX; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

The purpose of this study was to evaluate the influence of endothelial nitric oxide synthase (eNOS) deficiency on fetal growth, perinatal survival, and limb development in a mouse model with a targeted mutagenesis of the *Nos3* gene. Wild type (*Nos3^{+/+}*) and eNOS deficient fetuses (*Nos3^{-/-}*) were evaluated on gestational day (E) 15 and E17, newborn pups were observed on day1 of life (D1). For the evaluation of postnatal development, a breeding scheme consisting of *Nos3^{+/-}* x *Nos3^{+/-}* and *Nos3^{-/-}* x *Nos3^{-/-}* mice was established, offspring was observed for 3 weeks. Southern blotting was used for genotyping. No significant difference in fetal weight, crown-rump-lengths (CRL), and placental weight was seen between *Nos3^{+/+}* and *Nos3^{-/-}* fetuses on E15. By E17, *Nos3^{-/-}* fetuses showed significantly reduced fetal weights, CRL, and placental weights. This difference in body weight was also seen throughout the whole postnatal period. In pregnancies of *Nos3^{-/-}* females, the average number of pups alive on D1 was significantly decreased compared to either E15 or E17. Placental histology revealed no abnormalities. On E15, E17, and D1 *Nos3^{-/-}* fetuses demonstrated focal acute hemorrhages in the distal extremities among 0%, 2.6%, and 5.7%, respectively. Bone measurements showed significantly shorter bones in the peripheral digits of hindpaws of *Nos3^{-/-}* newborns. We conclude mice deficient for eNOS show characteristically abnormal prenatal and postnatal development including fetal growth restriction, reduced survival, and an increased rate of limb abnormalities. This characteristic phenotype of eNOS deficient mice dates back to the late third trimester of pregnancy.

Inherited Non-syndromic Cleft Palate in Mice: Phenotypic and Molecular Studies. *R.E. Blau*¹, *E.T. Everett*², *W. Harrison*³, *P.A. Overbeek*³. 1) DDS Program, Indiana Univ Sch of Dent, Indianapolis, IN; 2) Oral Facial Genetics, Indiana Univ Sch of Dent; 3) Dept of Cell Biology, Baylor Coll of Med, Houston, TX.

The secondary palate in mammals forms through a complex process and when perturbed can lead to cleft palate (CP). Clefts of the secondary palate are consistently included among the more common congenital anomalies occurring in humans, account for approximately one-third of all clefting cases, and carry an incidence of 3 to 9 per 10,000 livebirths / year. CP can be associated with more than 370 characterized disorders, yet more than 50% of CP cases occur as isolated and free of other anomalies (non-syndromic). Our long-term objectives focus on identifying and characterizing genetic determinants that contribute directly and/or indirectly to the occurrence of CP in humans. The identification of genes responsible for CP in mice will facilitate investigation of any putative roles played by their human homologs in familial and sporadic forms of CP. Four separate lines of transgenic mice resulting from recessive insertional mutations that cause non-syndromic CP have been identified. Chromosomal localization of the sites of transgene integration for three of these mutations has been performed. The mutant loci in the lines OVE270 and OVE1226B map to mouse chromosome 3 at bands B-C and F3, respectively. The transgene complex in the third transgenic line, OVE1328, maps to chromosome 4 at band A2. These regions share conservation of linkage with human chromosomes 1q21-q23, 1p13, 3q21-q26, 4q25-q31, and 8q11. Phenotypic studies of embryos from all four lines show cleft palate to be highly penetrant in homozygotes (tg/tg) with no additional overt dysmorphology or malformations present. In one line (OVE427) approximately 6% tg/+ embryos develop CP. Craniometric and radiographic studies of another line (OVE270) reveal mild craniofacial asymmetry and disturbances involving cranial base development. These disturbances of craniofacial growth and development in OVE270 tg/+ and OVE427 tg/+ mice may reflect effects of a haploinsufficient state. Finally, these mice will be invaluable tools for furthering our understanding of genetic factors associated with CP.

The Opitz syndrome gene product, MID1, interacts with the gene product of an X-chromosomal gene mapping to the linkage interval of FG syndrome. *S. Schweiger¹, A. Trockenbacher², V. Suckow¹, S. Krau¹, H.H. Ropers¹, R. Schneider².* 1) MPI for Molecular Genetics, Berlin, Germany; 2) Institute for Biochemistry, University Innsbruck, Austria.

Opitz syndrome is a genetically heterogeneous disorder characterized by defects of the ventral midline, including hypertelorism, cleft lip and palate, heart defects and mental retardation. We recently identified the gene responsible for X-linked Opitz syndrome (OS). The ubiquitously expressed gene product, MID1, is a member of the RING finger protein family characterized by an N-terminal tripartite protein-protein interaction domain. We could show that MID1 associates with microtubules through the proteins C-terminus and that mutant proteins found in OS patients abolish microtubules association and form cytoplasmic clots instead. In order to shed more light into the physiological function of MID1, we performed a yeast two hybrid screen. Using MID1 as the bait, we identified a novel binding partner of the MID1 protein, called MIDIA1. Two-hybrid results were confirmed by immunofluorescent experiments and co-immunoprecipitation of MID1 and MIDIA1 overexpressed in both COS7- and HeLa cells. Similar experiments using mutant MID1 proteins found in OS patients revealed that MIDIA1 is present in the cytoplasmic clots formed in the patients. These data implicate the loss or gain of MIDIA1 function as a potential factor in the molecular pathogenesis of OS. Chromosomal mapping assigned the MIDIA1 gene to the linkage interval of the FG syndrome on Xq13. FG syndrome is an X-linked recessive disorder characterized by agenesis of the corpus callosum, imperforate anus, facial abnormalities and similar symptoms that are related to the ventral midline. Proteins interacting with the MID1 protein are obvious candidates for other malformation syndromes involving the ventral midline, and mutation screening should soon clarify the role of MIDIA1 in the molecular pathogenesis of FG syndrome.

Gene expression changes in kidney stone disease. *E.G Tzortzaki¹, A.P Evan², S. Bledsoe², L. Deng¹, M. Yang¹, D. Glass¹, P.J Stambrook³, A. Sahota¹, J.A Tischfield¹.* 1) Department of Genetics, Rutgers University, Piscataway, NJ; 2) Department of Anatomy, Indiana University, School of Medicine, Indianapolis, IN; 3) Department of Cell Biology, Neurobiology and Anatomy, University of Cincinnati, College of Medicine, Cincinnati, OH.

A prominent feature of adenine phosphoribosyltransferase (APRT) deficiency is 2,8-dihydroxyadenine (DHA) nephrolithiasis. Using mRNA differential display, cDNA microarrays, and RT-PCR, we have recently identified changes in the expression of a subset of genes associated with chemotaxis, fibrosis, calcification, cell adhesion, and growth factors in a knockout mouse model for DHA lithiasis. As a first step in understanding the functional roles of these genes in the initiation and/or progression of kidney stone disease, we have used in situ hybridization (ISH) to identify their cellular locations in one- and three-month-old APRT-deficient mice. Digoxigenin-labeled oligonucleotide probes were hybridized to paraffin-embedded kidney sections. The sensitivity of ISH was demonstrated by detecting messages of low (APRT), medium (1,25-dihydroxyvitamin D3-24-hydroxylase gene), and high (kidney androgen-regulated protein, KAP) abundance. APRT and the selectable marker neo were used to demonstrate the specificity of expression. We also confirmed the proximal tubular localization of KAP and 24-hydroxylase. The expression of KAP was significantly reduced in APRT-deficient male mice, consistent with extensive tubular damage seen in these mice. The expression of imprinted multi-membrane spanning polyspecific transporter-like gene 1 (IMPT-1) was also decreased in proximal tubules, suggesting that impaired renal transport adversely affects renal function. The expression of matrix Gla protein (MGP), a developmentally regulated protein involved in tissue calcification, was significantly elevated in the cortex and inner medulla, suggesting a role for this protein in DHA-induced renal injury. Further characterization of these genes at different stages of disease progression will lead to a better understanding of the pathological basis of kidney stone disease. Supported by NIH grants DK38185, ES05652, and ES06096.

TBX5 is a cellular arrest signal during vertebrate cardiogenesis. C.J. Hatcher¹, M.-S. Kim¹, B. Wong¹, M.M. Goldstein², T. Mikawa¹, C.T. Basson¹. 1) Cardiology Division, Weill Medical College of Cornell University, New York, NY; 2) Division of Anatomic Pathology, Quest Diagnostics, Inc., Teterboro, NJ.

Mutations in human *TBX5*, a T-box transcription factor gene, are responsible for Holt-Oram syndrome, an autosomal dominant condition characterized by congenital cardiac septation and isomerism defects in the setting of limb deformity. We have used retroviral vectors to overexpress wildtype and mutant human *TBX5* isoforms *in vitro* and *in vivo* in order to determine the cellular function of *TBX5* in cardiac development. We observed that wildtype *TBX5* overexpression inhibits cell proliferation *in vitro*, and T-box binding to target DNA's major groove but not minor groove is necessary for this activity. Because human heterozygous *TBX5* mutations that impair T-box binding to target DNA's major groove most severely affect the heart, we hypothesized that *TBX5* participates in cardiogenesis by regulation of cell proliferation. Therefore, we overexpressed wildtype and mutant human *TBX5* isoforms in embryonic (E3) chick hearts *in vivo*. Despite mosaic *TBX5* overexpression, chick hearts infected with wildtype *TBX5* retrovirus were significantly smaller than control hearts; by E15, they exhibited decreased mass, thinned atrial walls and decreased ventricular trabeculation. In contrast, hearts infected with Gly80Arg-*TBX5*, a mutant isoform with disruption of the target DNA major groove binding site, exhibited no morphologic alterations. Immunostaining of genetically engineered chick hearts for PCNA, a nuclear proliferation antigen, revealed that *TBX5* overexpression suppresses myocyte proliferation *in vivo* via a major groove binding mechanism just as it does *in vitro*. *TBX5* inhibition of myocyte proliferation is not cell autonomous; we observed that wildtype *TBX5* overexpression inhibited proliferation of both infected and uninfected chick cardiomyocytes. Further immunohistochemical studies of human embryonic tissues also demonstrated that *TBX5* expression patterns are inversely related to cellular proliferation during human organogenesis. We, therefore, propose that *TBX5* acts as a cellular arrest signal during vertebrate cardiogenesis and thereby modulates growth and development of the human heart.

Asymmetric lateral plate and midline nodal expression in the proper development of laterality in the mouse

embryo. *M.R. Kuehn¹, L.A. Lowe¹, S. Yamada^{1,2}, T. Yamaguchi¹*. 1) Div. of Basic Sciences, NCI, NIH, Bethesda, MD; 2) Osaka University Faculty of Dentistry, 1-8 Yamadaoka, Suita, Osaka 565-0871, JAPAN.

The visceral organs of vertebrates develop along the left-right (L-R) body axis with handed asymmetry. For example, the heart and stomach are positioned on the left. During early somite stages the mouse nodal gene is expressed asymmetrically in the left lateral plate mesoderm (LPM). In addition midline expression of nodal around the node becomes asymmetric at these same stages. In mouse mutants with laterality defects, these asymmetric expression patterns are disrupted. Additional evidence implicates nodal in L-R development: ectopic right sided expression in chick or frog embryos leads to randomization of organ asymmetry. However, formal genetic proof and complete elucidation of nodal function in L-R development has been lacking because the null mutation causes early gastrulation stage defects. We have used a Cre/loxP strategy to generate a conditional mutant (floxed) allele of nodal. This floxed allele is also hypomorphic. Embryos heterozygous for the hypomorphic allele and a nodal null allele undergo gastrulation but display abnormal phenotypes at later stages. These defects fall into three distinct classes. One class of mutants specifically lacks left LPM expression of nodal, although expression around the node is seen. These embryos lack normal asymmetric expression of *Pitx2*, *lefty-1* and *lefty-2*. At later stages, these mutants exhibit random L-R asymmetry of the heart, transpositions of the great vessels, right isomerism of the lungs, and midline or reversed stomachs. These results provide conclusive evidence that nodal is essential for proper organ laterality, and give the first detailed insight into nodal's actual function. In further studies we have generated a transgenic strain that expresses Cre recombinase in the embryonic node, notochord and floorplate of the neural tube at stages overlapping nodal expression around the node. Crossing the Cre transgenic and floxed nodal lines will lead to deletion of the nodal locus only in cells in the node. This approach will allow us to assess the specific involvement of midline nodal expression in laterality determination.

Isn't one "zipper" enough to explain neural tube closure? Additional evidence to support the classic theory. *R.A. Saul¹, L.H. Seaver¹, J. Brown², R.E. Stevenson¹*. 1) Greenwood Genetic Ctr, Greenwood, SC; 2) Shriners Hospital for Children, Greenville, SC.

The classic view of human embryologic neural tube closure notes that closure starts just caudal to the rhombencephalon/cervical spinal cord junction (day 21) and proceeds rostrally to the anterior neuropore (day 26) and caudally to the posterior neuropore (day 28). Evidence from experimentally-induced neural tube defects (NTDs) in mice led to the multi-site closure model; that is, neural tube closure starts or occurs at five different sites (I,II,III,IV, and V). We have elected to study the caudal end of the neural tube by radiographic analysis of affected patients to investigate mechanisms of neural tube closure. We examined radiographs of the vertebral columns in 210 randomly selected patients seen at the Myelodysplasia Clinic at the Shriners Hospital in Greenville, SC from 1978 to 2000. We chose radiographs as a more direct measure of spinal dysraphism since external examinations might be biased by skin closure. Adequate analysis could be performed on 197 patients. All 197 patient radiographs demonstrated spinal dysraphism from the rostral end of the defect all the way to and through the sacrum. No discontinuous spinal dysraphism was observed. Our study calls into question closure site V. We see no radiographic evidence of caudal "closure" even when skin defects stop above the sacrum. Review of recent human embryos corroborates the classic view of neural tube closure (K. Sulik, 1999 and personal communication). While the multi-site closure model might explain externally identified varieties of NTDs (anencephaly [merocrania, holoacrania], encephalocele, faciocranioschisis, faciocraniorachischisis, craniorachischisis, and anencephaly with spina bifida), it is our suspicion that more thoroughly delineated cases (by thorough dissection and radiographic evaluations) will call into question the specificity of these closure sites. More thorough evaluations might blur the margins of these sites, leading to a re-evaluation of the processes and mechanisms involved in neural tube closure.

Mutation Screening of the Follicle Stimulating Hormone (FSH) Receptor Gene in Korean Infertile Men with Spermatogenic Defects and Hypergonadotropic Hypogonadism. *G.J. Song^{1, 5}, H.S. LEE¹, Y.S. Park¹, C.C. Lee⁵, Y.S. Lee^{2,4}, I.S. Kang^{3,4}.* 1) Laboratory of Reproductive Biology and Infertility, Samsung Cheil Hospital & Women's Healthcare Center, Seoul, Korea; 2) Department of Urology, Samsung Cheil Hospital & Women's Healthcare Center, Seoul, Korea; 3) Department of Obstetrics and Gynecology, Samsung Cheil Hospital & Women's Healthcare Center, Seoul, Korea; 4) Sungkyunkwan University School of Medicine, Seoul, Korea; 5) Department of Biology, Seoul National University, Seoul, Korea.

Follicle stimulating hormone (FSH) is considered to be important for spermatogenesis. Most infertile men with spermatogenic defects have elevated serum FSH concentration. Recently, an inactivating point mutation of the FSH receptor gene has been reported in men with elevated serum FSH concentrations and abnormal sperm parameters. In this study, we screened the point mutation of the FSH receptor gene using exon-specific PCR and SSCP analysis in 98 infertile men with severe hypospermatogenesis or azoospermia and high FSH concentration (>12 IU/l). From the results of SSCP analysis, abnormally shifted bands were detected in exon 10. Sequence analysis showed that either nucleotide A or G is frequently present at position 994 predicting the presence of a threonine or an alanine (AA genotype, 32%; AG genotype, 45%; GG genotype, 23%). However, this polymorphism was also found in the control group with a similar frequency (AA genotype, 35%; AG genotype, 48%; GG genotype, 17%). The FSH receptor genotype was not associated with serum FSH concentration and testicular volume. In conclusion, pathological mutation of the FSH receptor gene was not found in these Korean infertile men. It is suggested that mutation in the FSH receptor gene is not a common cause of male infertility.

New associations between prenatal exposure to drugs and malformations. *I.M. Orioli¹, E.E. Castilla²*. 1) Genetics, Fed. Univ. Rio de Janeiro, Rio de Janeiro, Brazil; 2) Genetics, FIOCRUZ, Rio de Janeiro, Brazil and CONICET, Argentina.

The aim of this work is to check known drug/malformation associations and search for new ones taking advantage of the database of the Latin American Collaborative Study of Congenital Malformations (ECLAMC) on drug exposure during the first trimester of pregnancy. In the ECLAMC the next non-malformed baby of the same sex born in the same hospital was selected as the healthy control subject for each malformed case. From the healthy newborn sample 15,378 infants whose mothers had taken one or more drugs during pregnancy were selected for this study. Likewise, 6,316 malformed infants having being exposed to drugs in utero were selected from 27 major, isolated congenital anomaly types. 19 specific drugs (i.e.: aspirine) or pharmacological groups (i.e.: antibiotics), according to sample size, were considered for analysis. Multiple logistic regression analyses were used with the characteristic of being or not affected by a certain anomaly as the dependent variable, while the 19 drug types were the independent ones. Maternal age, year of birth, and country of birth were considered as confounding factors. Several known and new associations were found. Abortives in general concentrated the higher number of positive associations with odds ratios higher than 3. Piperidolate was the drug more used by pregnant women in South America and showed an association with Poland complex. Other non described associations were: antialergics with intestinal, anal, and esophageal atresias; antiepileptics with cephalocele, micrognathia, ambiguous genitalia, equinovarus feet, and Poland complex ; aspirin with limb reduction; tuberculostatics with spina bifida, oral clefts, omphalocele, and intestinal atresias; thyroid hormones with limb reduction; anorexigens with spina bifida, cleft lip, and Adams complex; insulin/hypoglycemics with microcephaly, hypospadias, and intestinal atresias; sexual hormones with esophageal atresia and Adam complex; and corticoids with microcephaly and limb reduction.

Developmental Expression of Cytochrome P4501B1 (*Cyp1b1*). B.A. Bejjani¹, L. Xu², D. Armstrong¹, J.R. Lupski¹, L. Reneker². 1) Baylor Col Medicine, Houston, TX; 2) Univ of Missouri, Columbia, MO.

Homozygous and compound heterozygous mutations in *CYP1B1* have been shown to result in Primary Congenital Glaucoma (PCG). The purpose of this study is to analyze the expression patterns of *Cyp1b1* in the developing mouse ocular and brain tissues. Mouse tissues at different developmental stages were isolated for *in situ* hybridization. To make a riboprobe vector, a 646 base pair fragment from the 3'untranslated region (3'UTR) of *Cyp1b1* was amplified from mouse genomic DNA and inserted into the polylinker site of plasmid Bluescript. ³⁵S-labeled sense and antisense riboprobes were generated using an *in vitro* transcription kit. *Cyp1b1* is expressed in both anterior and posterior segments of the eye. Importantly, the expression in the anterior segment is confined to the ciliary body, most likely in the pigment ciliary epithelial cells. The earliest expression in the ciliary body is detected at postnatal day 4 (P4) and the expression continues into adulthood. Surprisingly, no detectable levels of *Cyp1b1* mRNA were found at or around the trabecular meshwork. In the posterior segments of the eye, *Cyp1b1* is expressed in the retina as early as embryonic day 12 and the expression becomes more specific to the inner nuclear layer by P7. Expression was also detected in the fat tissue around the optic nerve, but not in the optic nerve itself. We also found *Cyp1b1* mRNA in the meninges of the embryonic heads and in the hair follicles of the whiskers. It is hypothesized that developmental anomalies of the trabecular meshwork prevent appropriate drainage of the aqueous humor and cause PCG in human patients. Our results indicated that *Cyp1b1* expression is absent in this tissue in mouse eyes. Instead *Cyp1b1* is highly expressed in the developing and mature ciliary body. Our study suggests that ciliary body is involved in regulating the intraocular pressure either directly or indirectly by influencing the proper differentiation of trabecular meshwork.

Molecular bases of the phenotypic variability of lysinuric protein intolerance. *G. Sebastio¹, M.P. Sperandeo¹, V. Dall'Asta², O. Bussolati², G.C. Gazzola², M. Pineda³, M. Palacin³, V. Fiorito¹, A. Pietrosanto¹, M.T. Bassi⁴, G. Borsani⁴, A. Ballabio⁴, G. Andria¹.* 1) Dip. Pediatria, Federico II Univ, Naples, Italy; 2) Dip. Medicina Sperimentale, University of Parma, Italy; 3) Dep. Bioquímica y Biología Molecular, University of Barcelona, Spain; 4) TIGEM-HSR, Milan, Italy.

Lysinuric protein intolerance (LPI, MIM 222700), an autosomal recessive disorder of the y^+L transport of cationic amino acids, is caused by mutations of the SLC7A7 gene, which we recently identified. y^+L transport is exerted by the heterodimeric complex 4F2hc/SLC7A7, as seen by its expression in *Xenopus* l. oocytes. This activity is absent if the wild sequence of SLC7A7 is replaced by any of the 3 mutant alleles (M50K, S386R, W242X), found in Italian LPI patients. These data, though confirming the pathogenetic role of SLC7A7 in LPI, do not help to explain the extreme variability of the LPI phenotype. We also performed functional studies of y^+L in erythrocytes, fibroblasts and lymphoblasts from LPI patients and concluded that these cells normally express the y^+L transport activity. This suggests a genetic heterogeneity of y^+L transport. Being y^+L transport exerted by the heterodimeric complex 4F2hc/SLC7A7, we searched for the co-existence of mutations of 4F2hc in LPI patients as observed in compound heterozygotes for rBAT/SLC7A9 in non type-1 cystinuria, a disease with a molecular pathogenesis similar to LPI. However, we did not find any mutation of 4F2hc in Italian LPI patients, thus ruling out any role of 4F2hc in the determination of the LPI phenotype. Recently another gene, SLC7A6, has been shown to complex with 4F2hc and to yield a y^+L transport activity. We have elucidated the genomic structure of this gene which consists of 11 exons, spanning a region of some 60kb on chromosome 16q13. We are currently investigating a putative role of this gene in the pathogenesis of the variability of LPI phenotypes. Financial supports of MURST-PRIN 1998 (G.S and V.D.A.) and Telethon E.652 (G.S.) are gratefully acknowledged. M.P.S. is supported by Telethon- Italy (grant n.29cp) and is an Assistant Telethon Scientist.

Identification and characterization of a novel cochlear gene, *OTOR*, and localization of its encoded protein,

otoraplin. *N.G. Robertson*¹, *J.S. Lin*¹, *S. Heller*³, *B.L. Resendes*^{1,2}, *S. Weremowicz*^{1,2}, *C.S. Denis*³, *A.M. Bell*³, *J.C. Adams*^{2,4}, *A.J. Hudspeth*³, *C.C. Morton*^{1,2}. 1) Brigham & Women's Hosp, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Laboratory of Sensory Neuroscience and HHMI, The Rockefeller University, New York, NY; 4) Dept of Otology and Laryngology, Massachusetts Eye and Ear Infirmary, Boston, MA.

We identified a novel cochlear gene, designated *OTOR*, from a comparative sequence analysis of over 4,000 clones from a human fetal cochlear cDNA library. Northern blots of human and chicken organs show strong *OTOR* expression only in the cochlea; very low levels are detected in the chicken eye and spinal cord. *OTOR* localizes to chromosome 20 in bands p11.23-p12.1, and more precisely to STS marker WI-16380. cDNAs orthologous to *OTOR* were isolated from mouse, chicken, and bullfrog tissues. The encoded protein, designated otoraplin, has a predicted secretion signal peptide sequence and shows a high degree of cross-species conservation. Otoraplin is homologous to the protein encoded by *CDRAP/MIA* (cartilage-derived retinoic acid sensitive protein/melanoma inhibitory activity), which is expressed predominantly by chondrocytes, functions in cartilage development and maintenance, and has growth-inhibitory activity in melanoma cell lines.

We developed a polyclonal antibody against human otoraplin using a KLH-conjugated 16 amino acid-residue peptide from otoraplin's C-terminus as the immunogen. On western blots, the antibody specifically recognizes full-length otoraplin protein (~16 kDa). Preimmune sera and IgYs (from egg yolks of immunized chickens) do not show reactivity to otoraplin. Immunohistochemistry in human fetal inner ear shows presence of otoraplin in the organ of Corti, the spiral and vestibular ganglia, and in the macular epithelium. Immunostaining for otoraplin is also detected in the region of osteocytes in the surrounding temporal bone and in the modiolus, but not in chondrocytes in the same areas on fetal sections from earlier developmental ages.

Identification of a human melanoma inhibitory activity (MIA)-like gene which is specifically expressed in the human inner ear and exhibits a frequent polymorphism that abolishes protein expression. *N.D. Rendtorff¹, M. Frödin², T. Attié-Bitach³, J. Augé³, M. Vekemans³, N. Tommerup¹*. 1) Department of Medical Genetics, Institute of Medical Biochemistry and Genetics, the Panum Institute, University of Copenhagen, Copenhagen N, Denmark; 2) Department of Clinical Biochemistry, Glostrup Hospital, Glostrup, Denmark; 3) Department of Genetics and INSERM U-393, Hospital Necker, Paris, France.

Genes specifically expressed in the cochlea of the inner ear are candidate genes for non-syndromic hearing impairment. In order to discover new cochlea-specific genes, we searched in the TIGR database for clusters of ESTs, that have been isolated from the cochlea only. This led to the cloning and characterization of a human gene named MIA-like (MIAL) as it encodes a protein related to Melanoma inhibitory activity (MIA) protein, which is expressed and secreted by chondrocytes. The cDNA of the mouse homologue (Mial) was also cloned and mapped to mouse chromosome 2. By semi-quantitative RT-PCR, MIAL mRNA showed a very restricted distribution in humans as it was detected only in fetal inner ear and brain. In the mouse, Mial was expressed in the inner ear as well as in the brain, eye, limb and ovary. Using laser capture microdissection and in situ hybridization we show that MIAL is highly and specifically expressed in the sensory structures in inner ear of human conceptuses ranging from 24 days to 9,5 weeks. The MIAL cDNA predicts a protein of 128 amino acids including a signal peptide. In transiently transfected COS7 cells, the MIAL cDNA expressed a ~15 kDa polypeptide that was modified by sulfation and exists as a covalently linked homo-dimer of ~26 kDa. MIAL protein localized to the Golgi apparatus and was secreted into the culture medium. A frequent polymorphism was discovered in human MIAL. Of 505 individuals, 48 (9.5%) were heterozygous and 1 (0.2%) was homozygous. In COS7 cells, no protein synthesis was detected from cDNA of the variant allele, suggesting that human homozygotes do not express MIAL protein and that the variant MIAL allele may contribute to inner ear defects.

Genomic Struture, Chromosomal Localization and Functional Stuy of the Human Glycine N-methyltransferase

Gene. *Y.A Chen¹, L. Chen¹, J.V. Lin¹, T. Liu², T.L. Yang-Feng³*. 1) Prevention Medicine, Institute of Public Health, Taipei, Taiwan; 2) Department of Medical Research, Taipei Veterans GeneralHospital, Taipei, Taiwan; 3) Department of Genetics, Yale University, School of Medicine, New Haven, C.T. U.S.A.

Previously, we reported that the expression level of GNMT was diminished in human hepatocellular carcinoma (HCC). In this study, the human GNMT gene cloned and characterized. It contains six exons and spans about 10 kb (Accession No. AF101475). Instead of a TATA box, it has a transcriptional initiator located 801-bp upstream from the translation start codon. The gene was localized to chromosome 6p12 using FISH. The GNMT cDNA was cloned and placed under the control of the CMV promoter and the resultant plasmid was used to transfect HCC cell lines. The GNMT was found to be expressed in the cytoplasm of the HCC cells using IFA and it was translocated into the nuclei when the cells were treated with B(a)P. P-32 post-labeling and thin-layer chromatography were used to measure the B(a)P-DNA adducts. In comparison with HCC cells transfected with the vector plasmid, the B(a)P-DNA adducts formed in the cells transfected with pGNMT decreased at least 40% and the activation of cytochrome CYP1A1 enzyme activity by B(a)P was almost down regulated to the baseline level. Therefore, the expression of GNMT may protect the liver cells to against the insult of B(a)P.

Pyrin, the FMF Protein, Colocalizes with Microtubules in vivo and in vitro. *E.A. Mansfield¹, J.J Chae¹, H. Komarow¹, T. Brotz², M. Centola¹, D.L. Kastner¹.* 1) ARB/NIAMS, NIH, Bethesda, MD; 2) EIB/NCI, NIH, Bethesda, MD 20892.

Familial Mediterranean fever (FMF) is an autoinflammatory syndrome caused by recessively inherited mutations in MEFV; the microtubule inhibitor colchicine is highly effective in preventing FMF attacks. The product of MEFV, pyrin, is a protein of unknown function with a C-terminal RFP (B30.2) domain. Computational analysis of the sequence had suggested a possible role as a transcription factor. We examined the subcellular localization of pyrin in order to gain insight into its function. GFP and myc-tagged fusions of pyrin were expressed in Cos-7 and HeLa cells and the subcellular localization of the protein was examined by fluorescence microscopy. Transfected cells were double-stained with antibodies to various organelles and cytoskeletal proteins to determine if pyrin colocalizes with any cellular structure. These experiments demonstrated that pyrin does not occupy the nucleus, although in transfected cells, staining can be detected in the perinuclear region. All transfected cells show at least some staining throughout the cytoplasm. Cells with bright juxtanuclear staining were examined for colocalization of pyrin with the golgi apparatus. We did not observe colocalization of pyrin and golgi, although in some cells golgi and pyrin signals were adjacent. Treatment of transfected cells with brefeldin A disrupted the staining pattern of golgi but not pyrin. In contrast, treatment with the microtubule stabilizing drug paclitaxel dramatically altered the predominant staining pattern of pyrin, so that it colocalized prominently with bundled microtubules. Colchicine and nocodazole dispersed the pyrin signal in transfected cells into a diffuse or punctate pattern. We assayed the ability of pyrin to bind stabilized microtubules in vitro using both lysates of transfected cells and purified full-length recombinant pyrin. In both cases, pyrin was efficiently sedimented with the microtubule pellet. Deletion analysis showed that the N-terminal portion of pyrin is necessary and sufficient for microtubule binding. The function of the C-terminal RFP domain remains unknown at this time, although biochemical assays are underway to discover interacting proteins of pyrin.

Identification of six putative anion transporter genes in human and characterization of PAT1, a candidate gene for pancreatic anion exchanger. *H. Lohi*¹, *M. Kujala*¹, *E. Kerkela*², *U. Saarialho-Kere*², *M. Kestila*¹, *J. Kere*³. 1) Dept Medical Genetics, Haartman Inst, Helsinki, Finland; 2) Department of Dermatology, University Central Hospital, Helsinki, Finland; 3) Finnish Genome Center, University of Helsinki, Helsinki, Finland.

Members of the structurally well-conserved family of sulfate transporters have recently been identified as the second gene family of anion exchangers. The three known human members of this gene family, DTDST, CLD and PDS transport in different combinations, at least the chloride, iodine, bicarbonate, oxalate and hydroxyl anions. These genes are expressed in a highly tissue-specific manner and have all been linked to a different recessive disorder. We report the expansion of this gene family to include at least six physically mapped new members. We have characterized one of them, named PAT1 (for putative anion transporter 1), in more detail. It maps to chromosome 3p21.3, encodes a predicted 738 amino acid transmembrane protein, and is highly homologous to the known members of the family, suggesting its function as an anion transporter. PAT1 is most abundantly expressed in the kidney and pancreas. Pancreatic ductal cell lines Capan-1 and Capan-2 express PAT1 and immunohistochemistry localizes PAT1 protein to the apical surface of pancreatic ductal cells, suggesting it as a candidate for a luminal anion exchanger. The mapping and characterization of the novel members of this tissue-specific gene family may provide new insights to anion transport physiology in different parts of the body.

Approaches to study the genomic impact of human endogenous retroviruses. *D.L. Mager, J.R. Landry, L.N. van de Lagemaat, P. Medstrand.* Terry Fox Laboratory, BC Cancer Agency, and Dept. of Medical Genetics, Univ. of British Columbia, Vancouver, BC.

A remarkably high fraction of the human genome consists of retroelements that have become fixed in the species during the course of primate evolution. One class of retroelements is made up of the endogenous retroviruses (HERVs) and related solitary long terminal repeats (LTRs) which together comprise over 5% of human DNA. Because LTRs naturally contain transcriptional regulatory elements, we have been interested in determining how such elements can affect the expression of adjacent genes. Here we report on two genes, the apolipoprotein CI gene and the endothelin B receptor gene, for which a HERV-E LTR acts as an alternative promoter via splicing events. Fusion LTR-gene transcripts were initially detected through screens of the Expressed Sequence Tag database and promotion from within the LTR was confirmed by RT-PCR, RACE and/or primer extension. Interestingly, in the case of the endothelin B receptor, LTR-driven transcripts are detected only in placenta where they are comparable in level to the transcripts driven from the native promoter. Transient transfection experiments in the placental cell line JEG-3 using a reporter gene have shown that the HERV-E LTR at the endothelin B receptor locus is a strong promoter and can also enhance the activity of the native endothelin B receptor gene promoter and the SV40 promoter. This LTR has no significant activity in other cell types. As a complementary approach to examining the genomic effects of LTRs, we have developed computational tools for the analysis of integration patterns of many distinct LTR families and have applied these tools using the complete sequences of human chromosomes 21 and 22. Results suggest that LTR integration patterns are non-random with respect to distance along the chromosome and with respect to gene coding regions. These results support the hypothesis that endogenous retroviruses have contributed to the evolution of genes and genomes.

In silicon cloning of human myelin protein zero-like genes by bioinformatics strategy. *D. Tang^{1,2}, K. Yu², X. Tang², H. Zhang², Q. Pan², H. Dai², J. Xia².* 1) Department of Animal Science, Foshan University, Foshan, Guangdong, P R China; 2) National Lab Of Medical Genetics, Hunan Medical University, Changsha, Hunan, P R China.

To clone novel myelin protein related genes, two human ESTs, which shared significant similarity with the human myelin protein zero gene, were found by the comparison of homologue between the cDNA coding region sequences of MPZ gene and the EST database of NCBI. An 801 bp EST contig was assembled, which was 100% identical with a 128 kb genomic sequence, mapped to 1q24. A 435 bp open reading frame (ORF) within the 801 bp contig was shown by computer analysis. Two primers designed according to the sequence of the contig, were coupled with the primers (lgt10-5 and gt10-) on the sequences flanking cloning site of the cDNA library vector to amplify the cDNA library sequences by nested PCR. New primers, designed based on novel cDNA sequences, were used for the PCR amplification with lgt10-5 and gt10-5 in the same way as above. Finally, the human myelin protein zero like gene isoform I and II (MPZL1a, MPZL1b; GenBank: AF095727, AF092424) were cloned. Comparison of gene and protein structures between MPZL1 and MPZ revealed that MPZL1 is the second member of MPZ family. Mutation analysis of MPZL1 gene was performed in 24 Charcot-Marie-Tooth disease(CMT) families and 26 nonsyndrome deafness families, but no mutation was found.

Gene identification on chromosome 15q21-23 in a 200 Kb region spanning the CLN6 disease locus. *R.B. Wheeler¹, J.D. Sharp¹, R.A. Schultz², J.M. Joslin², B.D. Lake³, S.E. Mole¹, R.E. Williams¹, R.M. Gardiner¹.* 1) Department of Paediatrics, Royal Free and University College Medical School, UCL, London UK; 2) Eugene McDermott Centre for Human Growth and Development, University Texas South Western Medical Centre, Dallas, USA; 3) Department of Histopathology, Great Ormond Street Hospital for Sick Children, London, UK.

The neuronal ceroid lipofuscinoses (NCLs) are a group of autosomal recessive neurodegenerative diseases of childhood. CLN6 is the gene for a variant form of LINCL which we previously mapped to chromosome 15q21-23. The critical region was narrowed to approximately 200 Kb and two overlapping PACs from the region were sequenced at UTSWMC. This sequence, consisting of five contigs, together with additional sequence from the high throughput genome sequence databases (htgs) has been analysed using the NIX and Ensembl packages at HGMP (<http://www.hgmp.mrc.ac.uk>). Putative transcripts were identified by integrating the results of exon and gene prediction programmes eg. MZEF, GRAIL, GENSCAN and sequence similarity searches. Complete coding sequences were obtained by traditional methods. To date, two known genes, MEK5 and hnRNP core protein A1 and three novel genes have been identified. Candidate genes within the critical region have been analysed at the genomic and cDNA level in both human and the nclf mouse model but no mutations have been detected. However, several additional predicted genes and EST clusters are currently being analysed as candidates. Completion of the Human Genome Sequencing Project should lead to improvements in gene prediction programmes and facilitate future gene identification.

DAX1/SHP Family of the Nuclear Hormone Receptor Superfamily Appears Predisposed to Sequence Variability.

*M.V. Patel*¹, *G.B. Golding*², *Y.-H. Zhang*³, *B.-L. Huang*³, *A.P. Arnold*¹, *J.S. Sinsheimer*⁴, *E.R.B. McCabe*³. 1) Dept Physiological Science, UCLA, LA, CA; 2) Dept Biology, McMaster University, Hamilton, ON; 3) Dept Pediatrics; 4) Dept Biomathematics and Human Genetics, UCLA, LA, CA.

We have previously shown that DAX1 is predisposed to amino acid-changing substitutions similarly to SRY and greater than SOX9. The purpose of the current investigations was to compare DAX1 sequence changes during evolution with related genes: SHP, an autosomal encoded member, along with DAX1, of a family within the nuclear hormone receptor superfamily; SF1, a protein that interacts with DAX1; SOX3, a SOX member encoded on the X chromosome; SOX9, an autosomal SOX member involved in sex determination; and GK, a DAX1 neighbor in Xp21.3. We determined the genomic sequence of DAX1, SHP, SF1, SOX3, SOX9, and GK in the rhesus macaque, and compared these with genomic sequences in human and mouse. The number of non-synonymous substitutions per non-synonymous site ($K_a \times 100$) was calculated for rhesus/human pairwise comparisons. K_a values are DAX1=1.1 \pm 0.32; SHP=0.970 \pm 0.34, SF1=0.57 \pm 0.23, SOX3=0.30 \pm 0.18, SOX9=0.00 and GK=0.00. For rhesus/mouse comparisons, codon positions 1 and 2 of DAX1 show a high percentage of transversions (0.096 and 0.090) and transitions (0.12 and 0.081), supporting the high K_a value for DAX1. In contrast, changes in the 3rd position are not significantly different than the other genes. DAX1 also shows differential codon preferences. Rhesus/mouse DAX1s show differences in four amino acids: Ala (GCG vs. GCC), Arg (CGG vs. AGA), Asp (GAC vs. GAU), and Thr (ACG vs. ACC). SHP and GK have differences in two amino acids, and SF1, SOX3 and SOX9 show identical codon preferences for all amino acids. We then examined the percentage of nonsynonymous codons differing in two out of three or all three positions in rhesus/mouse comparisons: 13.4% of the amino acid changing codons in DAX1 and 11.2% in SHP change in more than one position, compared with 2.6%, 2.44%, 1.38% and 0.76% for SF1, SOX3, SOX9, and GK, respectively. We conclude that, according to several measures, DAX1 is quite variable at the protein level, and SHP, a family member of DAX1, appears to share this predisposition for variability.+++.

Loss-of-function mutations in DAP12 in PLOSL patients provide a new link between the immune system and dementias. *J. Paloneva*^{1,2}, *M. Kestilä*³, *J. Wu*⁴, *A. Salminen*³, *T. Böhling*³, *V. Ruotsalainen*⁵, *P. Hakola*³, *A.B.H.*

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PLOSL (Polycystic Lipomembranous Osteodysplasia with Sclerosing Leukoencephalopathy; MIM 221770), also known as Nasu-Hakola disease, is a recessively inherited disease characterized by a unique combination of psychotic symptoms rapidly progressing to presenile dementia and bone cysts restricted to wrists and ankles. The first symptoms appear around age 20 and the disease results in death by the age 50. PLOSL has a global distribution, although most of the patients have been diagnosed in Finland and Japan, with an estimated population prevalence of 2×10^{-6} in the Finns. We have mapped the PLOSL-locus to 19q13.1 and identified a shared 153-kb ancestor haplotype in all Finnish disease alleles. Here we report the molecular defect in PLOSL by identifying one large deletion in all Finnish PLOSL alleles and another mutation in a Japanese patient, both representing loss-of-function mutations, in the gene encoding DAP12 (TYROBP). DAP12 is a transmembrane protein that has been recognized as a key activating signal transduction element in natural killer (NK) cells. On the plasma membrane of NK cells, DAP12 associates with several activating receptors recognizing major histocompatibility complex (MHC) class I molecules. Interestingly, no abnormalities were detected in the number or function of the NK cells in PLOSL patients, homozygous for a null allele of DAP12. Instead, they display pathology in the central nervous system (CNS) and bone. This finding not only provides new clues to the pathogenic mechanisms of dementias, but should also stimulate a search for novel biological functions of the DAP12 molecule.

Hfb1 brainspecific sequence is the 3'-UTR of the complexin 2 mRNA. *L.V. Dergunova¹, N.M. Raevskaya¹, I.P. Vladychenskaya¹, A.B. Poltarau², S.A. Limborska¹.* 1) Dept Human Molecular Genetics, Inst Molecular Genetics, Moscow, Russia; 2) Institute of Molecular Biology, Moscow, Russia.

Brain-specific Hfb1 sequence was obtained from human forebrain cortex cDNA library and its primary structure was determined (Acc. No Y15167). By Northern blot hybridization analysis the size of the full length transcriptional product corresponding to Hfb1 was estimated to be about 5,0 kb. Hfb1 was mapped to the human chromosome 5. Human chromosome 5 cosmid library was screened and Hfb1- positive clone with the insert more than 20 kb was selected. 4,5 kb genomic fragment gHfb1 corresponding to Hfb1 was sequenced. Comparison to the sequences of the GenEMBL database using the BLAST program has revealed the 56 nucleotides overlapping region between gHfb1 5'-end and human complexin 2 mRNA 3'-end. The overlapping is supported by two additional ESTs AL119131 and AW896858 highly homologous both to gHfb1 and complexin2 mRNA. These results suggest gHfb1 to be complexin 2 3'-untranslated region. The complexin 2 protein is known to take part in the synaptic vesicle transport processes and is encoded by 940 nucleotide mRNA (Acc. No NM 006650) with the reading frame between 346 and 750 nucleotides. We estimate the size of complexin 2 mRNA 3'-UTR to be about 4 kb . The computer comparison has also shown a large number of short unknown ESTs highly homologous to gHfb1 with their 3'-ends coincident to Hfb1 3'-end and the absence of any homologous ESTs with 3'-ends extending Hfb1 last nucleotides thus confirming gHfb1 to be the part of the last exon of the complexin 2 gene.

Mice trisomic for a BAC with the single minded 2 gene (Sim2) show phenotypes similar to some of those present in the partial trisomy 16 mouse models of Down syndrome. *R. Chrast¹, H.S. Scott¹, R. Madani², L. Huber², D.P. Wolfer², M. Prinz³, A. Aguzzi³, H.P. Lipp³, S.E. Antonarakis¹.* 1) Division of Medical Genetics, Geneva University Medical School and University Hospital, Geneva, Switzerland; 2) Institute of Anatomy, University of Zurich, Switzerland; 3) Institute of Neuropathology, University Hospital of Zurich, Switzerland.

The *Drosophila* single minded (*sim*) transcription factor, is a master regulator of fruitfly neurogenesis. Recently, we have cloned and mapped a human homologue of *sim*, *SIM2*, to chromosome 21 in the so called "Down syndrome critical region". Three copies of *SIM2* may contribute to some Down syndrome (DS) phenotypes because of the mapping position, function as transcriptional repressor, temporal and spatial expression pattern of mouse *Sim2*, and the potentially analogous role of human *SIM2* to that of *Drosophila sim* during neurogenesis. In order to validate this hypothesis *in vivo*, we have created the first BAC transgenic mice overexpressing a gene possibly involved in DS with only one or two additional copies of mouse *Sim2*. The transgene was shown to be expressed in the same spatial pattern as the endogenous gene. The mice develop normally, are fertile and do not show readily detectable histopathological abnormalities. However, detailed analysis of their behavior revealed changes in their exploratory behavior, and sensitivity to pain, phenotypes which are also present in other partial trisomy 16 mouse models of DS. Our data therefore suggest that overexpression of *SIM2* contributes to some of the complex DS phenotypes.
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Evolutionary conservation and differential expression of *EPM2A* gene. *S. Ganesh*¹, *K. Shoda*¹, *A.V. Delgado-Escueta*², *K. Yamakawa*¹. 1) Laboratory for Neurogenetics, RIKEN Brain Science Institute, Wako-shi, Saitama, Japan; 2) Comprehensive Epilepsy Program, UCLA , Los Angeles, California.

The progressive myoclonus epilepsy of Lafora type is an autosomal recessive disorder caused by mutations in the *EPM2A* gene encoding a putative tyrosine phosphatase. We isolated full-length transcripts of human, mouse and rat orthologues and compared their expression profile. Sequence comparison reveals a highly conserved laforin, the predicted protein product of *EPM2A*. Rat orthologue shows 94 and 95% similarity to human and mouse laforin respectively and in all the three species, the estimated molecular size of laforin is approximately 38 kDa. Interestingly, all the missense mutations identified in Lafora disease families so far were substitutions for highly conserved residues suggesting that laforin's function may have been altered in those patients. *EPM2A* is ubiquitously expressed in various adult tissues of human, rat, and mouse but in human brain, its expression level was notably lower. In mouse, transcripts were detected from 7-dpc embryonic stage onwards and the expression level was higher in adults. We identified differentially spliced transcripts for human *EPM2A* and one of them encodes a truncated laforin. Our results suggest the presence more than one promoter for *EPM2A* in human and that the multiple isoforms may be unique to human. To understand the function of laforin and its isoforms, antibodies were generated for specific regions and studies are underway to characterize the protein using in vivo and in vitro model systems.

Mapping of alpha6 nicotinic cholinergic receptor subunit (CHRNA6) gene on chromosome 8p11 and analysis of the gene structure. *M. Ebihara, H. Ohba, S. Ohno, T. Yoshikawa.* Laboratory of Molecular Psychiatry, Brain Science Institute, Riken, Saitama, Japan.

There are eight alpha subunits (alpha2-alpha9) and three beta subunits (beta2-beta4) in the nicotinic cholinergic receptor gene family. The nicotinic cholinergic receptors are multimeric ligand-gated ion channels and involved in neuronal functions. Recently it has been reported that autosomal dominant nocturnal frontal lobe epilepsy is caused by mutations in the alpha4 subunits of the nicotinic cholinergic receptor gene, although the mice lacking nicotinic cholinergic receptor (i.e. CHRNA4, CHRNA7, CHRNB2) did not show any neurological defects. In 1999, postnatal growth deficiency, megasystis and mydriasis have been reported in mice lacking the alpha3 nicotinic cholinergic receptor gene. Thus it is important to study the function of the nicotinic cholinergic receptors in order to understand various phenotypes linked to the receptors, especially epileptic disorders. In this study, we analyzed the gene structure and function of the alpha6 nicotinic cholinergic receptor including the promoter region. CHRNA6 consists of six exons, spanning approximately 10kb. The alpha6 nicotinic cholinergic receptor gene is mapped on chromosome 8p11 by FISH.

Characterisation of the human homologue of a candidate gene for Slow Wallerian Degeneration. *S. Fernando*¹, *S. Tosi*², *L. Conforti*¹, *A. Tarlton*¹, *E.A. Buckmaster*¹, *V.H. Perry*¹, *A.D. Smith*¹, *M.P. Coleman*¹. 1) Pharmacology, University of Oxford, Oxford, UK; 2) Institute of Molecular Medicine, Oxford, UK.

Wallerian degeneration is the process by which the distal part of a nerve axon degenerates following injury. Slow Wallerian degeneration in which this process is delayed has been observed in a mutant strain of mouse, C57BL/Wlds, and in this mouse candidate genes were identified within an 85Kb tandem triplication unit on distal mouse chromosome 4. The proximal and distal boundaries of this triplication unit are spanned by the gene encoding ubiquitin fusion degradation protein 2 (Ufd2) and a previously undescribed gene, D4Cole1e respectively. A third gene within the triplication unit, Rbp7, is not a good candidate for Wlds. Ufd2 and D4Cole1e produce a chimeric mRNA in the Wlds mouse which encodes an in-frame fusion protein. The protein consists of 70 amino acids from the N-terminal region of Ufd2 and 302 amino acids from the C-terminal region of D4Cole1e and is abundantly expressed in the nervous system. As Wallerian degeneration is the end-point of many forms of axonal pathology in the central and peripheral nervous systems the human homologues of these genes could influence susceptibility to neurological disorders. We have identified the human homologue of D4Cole1e, which we have mapped to chromosome 1p36.2 by fluorescent in-situ hybridisation. Northern blot analysis has shown that it is abundantly expressed in the human brain. The exons corresponding to D4Cole1e gene have been cloned and sequenced. A search is on-going for Single Nucleotide Polymorphisms within these exons in order to investigate whether this gene influences the phenotype of neurodegenerative diseases in humans.

ApoE and APP are alternative ligands for the calcium-sensing receptor. *Y.P. Conley^{1,2}, D.N. Finegold², R.E. Ferrell².* 1) Department of Health Promotion & Development, University of Pittsburgh, Pittsburgh, PA; 2) Department of Human Genetics, University of Pittsburgh, PA.

The calcium-sensing receptor (CaSR) is a G-protein coupled receptor primarily involved in systemic calcium homeostasis. The CaSR is expressed in tissues involved with calcium homeostasis such as the parathyroid and kidney, however, the CaSR is also expressed in tissues where its function is not readily apparent, for example the brain. We hypothesized that the brain CaSR might have a function other than mineral ion metabolism that could be explained by alternative ligands for the receptor. We conducted sensitive luciferase reporter gene assays to determine if the CaSR is activated by Apolipoprotein E (ApoE) isoforms and amyloid precursor protein (APP), substances implicated in neurodegeneration. We demonstrated that the CaSR is significantly activated by these ligands when compared to a control reaction where no ligand is added to the cultures. APP activated the receptor 36% over the control ($p < 0.001$). ApoE3 activated the receptor 49% over the control ($p < 0.01$). ApoE4 activated the receptor 39% over the control ($p < 0.001$). ApoE2 activated the receptor 19% over the control; however the results did not reach significance. ApoE and APP have been implicated in alterations of intracellular calcium concentration via a mechanism that is currently unknown. ApoE is known to increase cytoplasmic calcium via the phospholipase C pathway and APP is involved with oscillations in intracellular calcium concentration in functional synapse formation. ApoE and APP activation of the CaSR could explain the mechanisms that ApoE and APP utilize to alter intracellular calcium levels and support the calcium hypothesis in neurodegeneration.

The modulation of HIP1 function through its interaction with its family member HIP12: implications for the pathogenesis of Huntington disease. *V. Chopra¹, M. Metzler¹, D. Rasper², R. Singaraja¹, L. Gan¹, D. Nicholson², M. Hayden¹.* 1) Department of Medical Genetics, and Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, Canada, V5Z 4H4; 2) Department of Biochemistry and Molecular Biology, Merck Frosst Centre for Therapeutic Research, Montreal, Canada, H9R 4P8.

Huntingtin-interacting protein 1 (HIP1) was identified through its interaction with huntingtin, the polyglutamine-containing protein altered in Huntington Disease. Although the function of HIP1 is unknown, it shows homology to Sla2p in *Saccharomyces cerevisiae*. Sla2p is essential for endocytosis and the assembly and function of the cytoskeleton in yeast. Recent evidence suggests that huntingtin may play a potential role in vesicle transport. These findings suggest that the interaction between HIP1 and huntingtin is necessary for vesicle trafficking and maintenance of the membrane cytoskeleton. As such, the altered interaction of HIP1 with huntingtin could result in a disruption of intracellular transport leading to neuronal cell death. Interestingly, it has recently been demonstrated that HIP1 contains a death effector domain (DED). Furthermore, HIP1 has been shown to be toxic when overexpressed in human embryonic kidney (HEK) 293T cells. We have identified a novel protein termed HIP12 that shows profound sequence and biochemical similarities to HIP1 and also shows high sequence similarity to Sla2p, indicating that HIP1 is a member of a family of cytoskeletal-associated proteins. HIP1 and HIP12 show similar subcellular distribution in both neuronal and non-neuronal cell types and are both found within the striatum, the brain region affected in HD. Using the yeast 2-hybrid system and co-immunoprecipitation studies we have demonstrated that HIP12 does not interact with huntingtin but can bind directly with HIP1. Furthermore, HIP12 does not confer toxicity when overexpressed in HEK 293T cells but can reduce the toxicity of HIP1 when co-expressed in the same cells. These findings suggest that the potential interaction of HIP12 and HIP1 in vivo may influence the function of each respective protein and this may have important implications in HD.

Down Syndrome Cell Adhesion Molecule: An endogenous promoter element drives the expression of a candidate gene for DS Congenital Heart Disease in the developing mouse embryo. *G.M. Barlow¹, X.-N. Chen¹, G.E. Lyons², J.R. Korenberg¹*. 1) Cedars-Sinai Medical Center, Los Angeles, CA; 2) University of Wisconsin Medical School, Madison, WI.

Down Syndrome (DS) is a major cause of congenital heart disease (CHD), and is normally caused by trisomy for chromosome 21. Through the study of rare individuals with partial trisomy 21 and DS-CHD, we have narrowed the candidate region for DS-CHD to D21S3 through the PFKL. DSCAM (Down Syndrome Cell Adhesion Molecule) spans more than 840 kb of this narrowed DS-CHD region and encodes a highly conserved member of the immunoglobulin superfamily. The cardiac features of DS implicate disturbances in cell adhesion as responsible for DS-CHD. Further, using the collagen gel system as a model, we have shown that disruption of DSCAM expression using antisense oligonucleotides inhibits the transformation and/or migration of mesenchymal cells derived from the AV cushions. We now report the identification of a 1.8 kb region with DSCAM promoter activity. A reporter construct with this putative promoter region driving lac Z gene expression was generated and introduced into fertilized mouse eggs by pronuclear injection. The resulting mouse embryos were harvested at E12.5 and analyzed for the expression of the lac Z transgene by staining with X-gal. Several embryos showed strong lac Z expression in the ventral diencephalon, in the choroid plexus of the fourth ventricle and in the floor of the rhombencephalon. Specific staining was also observed in subsets of cells of Rathkes pouch and in the Gasserian, superior cervical and dorsal root ganglia. One embryo also showed transgene expression in the skeletal condensations of the developing fore- and hind-limbs. These results represent a subset of the DSCAM expression previously observed by tissue in situ hybridization (TISH) and are consistent with a role for DSCAM in the developing nervous system and limb bud.

Laforin in Lafora's progressive myoclonus epilepsy: Function and subcellular localization. *B.A. Minassian^{1,2}, L. Ianzano¹, D. Molinari de Andrade¹, E.J. Young¹, E. Chan¹, C.A. Ackerley³, S.W. Scherer¹.* 1) Dept of Genetics, Hosp Sick Children, Toronto, Canada; 2) Div of Neurology, Hosp Sick Children, Toronto, Canada; 3) Dept of Pathology, Hosp Sick Children, Toronto, Canada.

Lafora's disease (LD) is an autosomal recessive neurodegenerative condition with onset in teenage years and death within 10 years of onset. Two striking features characterize LD: A severe increasingly intractable epilepsy dominates the clinical course, and pathognomonic perikaryal polyglucosan inclusions are seen in neurons and other tissues.

We recently identified the LD gene, EPM2A (protein product named laforin). Transcript and mutation analysis revealed 4 essential exons comprising the consensus gene, but several alternative transcripts were also identified. Laforin's C-terminus (coded by exon 4) demonstrates high sequence identity with the catalytic domains of dual-specificity phosphatases. Laforin also appears to contain putative carbohydrate binding and glucosylhydrolase domains.

In order to study potential phosphatase activity, several bacterially expressed GST-laforin fusion proteins were assayed using the phosphatase substrate pNPP. Whereas wild-type laforin readily dephosphorylated pNPP, mutants including exon 4 alone, exons 2-4, or a single amino acid change (C271S) at the putative catalytic cysteine exhibited no phosphatase activity.

In order to determine the subcellular localization of laforin, COS cells were transfected with Myc epitope-tagged laforin. In-situ hybridization and electron microscopic analyses revealed two sites of localization, at the internal aspect of the plasma membrane and in a perikaryal distribution colocalizing with the endoplasmic reticulum (ER). Results with the Myc tag placed at the N or C terminus were identical.

Further studies are underway to unravel laforin's functions including its potential to bind and hydrolyze the perikaryal polyglucosans and its role at the neuronal plasma membrane.

Circadian output pathway genes from mouse liver. *K. Kaasik, C.C Lee.* Molecular and Human Genetics, BCM, Houston, TX.

Circadian clocks control behavior and physiological processes in mammals and in many other organism. The suprachiasmatic nucleus is the central clock structure in mammals. However recent findings indicate that clock elements have found in many of the peripheral tissues in mammals. To better understand the significant of that peripheral clock mechanism we undertook a study to identify genes under the control of peripheral clocks. To find candidate genes we make used of the observation that expression of circadian clock control genes cycle in a daily fashion and the detection of these expression changes by cDNA microarray analysis. To date by analyzing 1000 UniGene library cDNAs using cDNA probes generated from mouse liver mRNA from different circadian time points we have identified several clones that display apparent differential diurnal expression. The differences in expression level between CT 0 and CT 12 were at least 5 times using cDNA microarray analysis. To confirm the microarray analysis, Northern blot using mRNA from wild type and different clock mutant mice is currently in progress.

Expression analysis of gene from human chromosome 21. *D. Slavov*¹, *M. Davisson*², *L. Bechtel*², *K. Gardiner*¹. 1) Eleanor Roosevelt Inst, Denver, CO; 2) The Jackson Laboratory, Bar Harbor, ME.

Analysis of the recently completed sequence of human chromosome 21 identified 225 genes and gene models. Analysis of the predicted protein sequences shows that ~100 genes/models so far have no similarity to known proteins and no identifiable functional domains or motifs. Furthermore, of the ~125 genes with some functional association, none has been linked to a clear role in the Down syndrome phenotype. Because functional data are limited, expression data may be the most efficient basis for selecting genes for further study of relevance to Down syndrome. Because these data are inadequate or completely lacking for the majority of chromosome 21 genes, we are focussing on the following approaches: i) Based on ESTs and exon prediction and using RT-PCR and RACE, we have generated complete or near complete coding regions for a ~20 novel gene models. ii) dbEST analyses and semi-quantitative RT-PCR have been used to determine expression and alternative processing in ~30 tissues and cell lines for >40 known and novel genes/models; iii) quantitative Northern and RT-PCR analysis using normal and Down syndrome derived cell lines and tissues from normal mice and the Down syndrome mouse model, Ts65Dn, are being used to determine trisomy-associated changes in expression levels; iv) RNA tissue in situ is being carried out on mouse neonatal brain for a subset of known and novel genes. Interesting results include very low levels of expression of many novel genes, a high frequency of potentially functionally relevant alternative processing, gene and tissue specific increases in expression level that range from 0 to 3-5 fold in trisomies, and a set of candidate genes with hippocampal expression.

Molecular genetics of the human very-low dopamine b-hydroxylase (DBH) trait. *C.P. Zabetian^{1,3}, G.M. Anderson², J. Gelernter^{1,3}, H. Ichinose⁴, T. Nagatsu⁴, R.T. Malison¹, J.F. Cubells^{1,3}.* 1) Dept of Psychiatry, Yale Univ, New Haven, CT; 2) Child Study Ctr, Yale Univ, New Haven, CT; 3) Dept of Psychiatry, VACHS, West Haven, CT; 4) Inst Comp Med Sci, Fujita Health Univ, Toyoake, Japan.

Dopamine b-Hydroxylase (DBH) catalyzes the final step in the biosynthesis of norepinephrine, a neurotransmitter involved in many diseases with significant heritability including psychiatric and substance use disorders, Parkinson's disease, and hypertension. DBH enzyme activity is readily assayed in blood, highly heritable, and strongly linked to the DBH gene. Activity values are bimodally distributed; 3-4% of the European-American (EA) population exhibits very low DBH levels (DBH-L), apparently transmitted as a recessive Mendelian trait. The goal of this study was to identify a functional polymorphism(s) responsible for DBH-L. DNA was collected, and DBH activity measured, in EA and Japanese (JP) subjects. DBH-L, average-, and high-DBH individuals were screened by sequencing the proximal 1kb of the promoter, all 12 exons, and all intron/exon boundaries of the DBH gene. Novel single nucleotide polymorphisms (SNPs), and all previously described nonsynonymous SNPs were then genotyped in a larger group of samples. No amino acid sequence-altering SNPs were associated with DBH-L. No new exonic or promoter polymorphisms were found, but a number of unreported intronic SNPs were identified. One such SNP was highly correlated with the very-low DBH trait. All EA and JP DBH-L individuals were homozygous for the same allele. Whether this SNP is itself functional, altering splicing efficiency for example, or simply in linkage disequilibrium with an as yet unidentified functional polymorphism is unknown. Supported by: USDVA, NIDA, NIAAA, NARSAD.

Mutants in two DNA polymerases, *REV3(z)* and *RAD30(h)* differ in cross-link repair. *K.F. Grossmann, A.M. Ward, M.E. Matkovic, J.A. Hejna, R.E. Moses.* Molec & Med Genetics, Oregon Hlth Sci Univ, Portland, OR.

The human autosomal recessive disease Fanconi Anemia (FA) displays a cellular phenotype of specific hypersensitivity to DNA cross-linking agents and a prolonged arrest in G2 after cross-linking damage. *S. cerevisiae* cells with a deletion in the catalytic subunit of a translesion DNA polymerase, REV3, are also sensitive to cross-link damage, while not significantly sensitive to UV light or Angelicins, much like FA cells (Grossmann, *et al*, Mutation Research, in press). Additionally, *rev3* mutants display a terminal arrest in G2 after CDDP damage, unlike wild-type cells, suggesting that the Rev3 DNA polymerase might play a role in the post-replication repair of DNA cross-links. Rad30, the yeast homolog to human xeroderma pigmentosum variant (XP-V), is a DNA polymerase representative of a new class of DNA polymerases with translesion synthesis function. We find that *rad30* mutants, while sensitive to UV, are not sensitive to CDDP and have no prolongation of G2 arrest after CDDP treatment compared to wild-type cells, suggesting that, unlike Rev3, this bypass polymerase does not play a significant role in post-replication cross-link repair. Our results indicate that DNA polymerases demonstrated to have bypass properties have different specific roles in DNA repair and that the XP-V homolog does not appear to be required for cross-link repair in yeast.

Inhibition of cell proliferation by the TSC1 and TSC2 tumor suppressor genes. *L. Khare, A. Astrinidis, P.D. Adams, E.P. Henske.* Dept Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA.

Tuberous sclerosis complex (TSC), a tumor suppressor gene disorder, is characterized by tumors of the brain, heart, and kidney. TSC is caused by mutations in two genes, TSC1 and TSC2. Unlike TSC2 which has been studied more extensively, there have been no studies to investigate the relationship between TSC1 and the cell cycle. We have overexpressed TSC1 in various cell lines to observe its effect on growth and cell cycle kinetics. Hamartin, the TSC1 product, has been found to have a direct interaction with the TSC2 product, tuberlin, suggesting that tuberlin and hamartin function in the same molecular pathway. TSC2 has been shown to suppress growth of Rat1 fibroblast cells and cells lacking TSC2 derived from the Eker rat, a model of TSC2-linked disease. TSC2 has also been shown to cause an elongated G1 phase in Eker rat embryonic cells, Rat1 fibroblast cells and human neuroblastoma cells. We studied the effect of overexpression of TSC1 and TSC2 in several cell lines. We found that overexpression of TSC1 and/or TSC2 resulted in a suppression in the colony forming ability of MCF-7 and HeLa cells, indicating that both genes have a growth suppressing effect. We then used flow cytometry to analyze the cell cycle profile of 293 kidney cells and HeLa cervical adenocarcinoma cells overexpressing TSC1 and/or TSC2. Overexpression of TSC2 in 293 kidney cells resulted in increased proportion of cells in G1 fraction of the cell cycle. Overexpression of TSC1 did not lead to an arrest of the cell cycle in the G1 phase. This is the first report showing that TSC1, like TSC2, has a cell growth suppressing activity as observed in the colony formation assay, but this effect may not be mediated by a G1 arrest in the cell cycle.

Promoter Analysis of TBX5. *S.J. Cross, A.J. Bonser, T.K. Ghosh, J.D. Brook.* Institute of Genetics, University of Nottingham, Queens Medical Centre, Nottingham, United Kingdom.

T-box genes are a family of developmentally-important transcription factors related to mouse Brachyury. Mutations in one member of the family, TBX5, have previously been shown to cause Holt-Oram syndrome, a disorder of the development of the heart and skeleton. Holt-Oram patients typically present with septal and conduction defects of the heart, and with shoulder and radial malformations. The promoter region of TBX5 has yet to be described, and the precise length of the transcript is not known. Also, little is known about the regulation of this gene in the developmental pathways within which it acts. In an attempt to fill these gaps in current understanding, we have sequenced extensively from a genomic clone of this region and have used a variety of sequence analysis programs to predict the location of the transcriptional start and promoter of TBX5. We have also identified sites for the binding of transcription factors involved in heart and limb development in the upstream region of TBX5. Reporter assays in tissue culture have been used in order to dissect the functional elements of the promoter of this gene. We present here a map of a total of approximately 13kb of the upstream region of TBX5, showing the relative positions of important promoter elements, along with an details of a new gene with exons located within the promoter of TBX5.

Functional analysis of human thymosin b4 promoter in aortic valve interstitial cells. *Q.Y. Li, R.P. Lafferty, R.J. Levy.* Cardiology Res/Abramson Bldg, Children's Hosp Philadelphia, Philadelphia, PA.

Calcific aortic valve stenosis is the most common cardiac valvular disorder. Studies have showed that abnormal extracellular matrix (ECM) remodeling, for example, the deposition of bone related proteins, is associated with the progression of aortic valve calcification. Recently, we observed that an ECM protein involved in bone development, tenascin C (TN-C), is co-localized with metalloproteinase-2 (MMP-2) in the perimeter of calcified nodules in human aortic valve. In order to understand the disease-associated ECM remodeling, we established sheep aortic valve interstitial cell culture. We found that TN-C can induce MMP-2 expression in these cells. Using cDNA subtraction and competitive RT-PCR, we identified thymosin b4 (Tb4) as a downstream gene upregulated by TN-C. Furthermore, we observed that Tb4 upregulates MMP-2 expression in sheep and human aortic valve interstitial cells. Thus, we decided to study the promoter function of Tb4 in aortic valve interstitial cells in order to understand how Tb4 gene is regulated by TN-C. Human Tb4 belongs to a multiple gene family. By PCR screening a human PAC genomic library, we identified five PAC clones carrying Tb4 conserved sequences. We also cloned the Tb4 cDNA expressed in human aortic valve interstitial cells. In comparisons of the cDNA sequence and the sequences derived from PCR products of the PAC clones, we identified two PAC clones that share 96-97% identity with the Tb4 cDNA. We isolated the restriction fragments with Tb4 by Southern blot and subcloned them into pBluescript vector. The 5' untranscribed regions of Tb4 genes from these PAC are cloned into pGL-Basic (promoterless luciferase gene). The promoter activities of these promoter-reporter constructs are being examined in both sheep aortic valve interstitial cells cultivated on type I collagen with or without TN-C. The promoter showing induction activity for TN-C will be truncated for further functional analysis in order to identify the minimal TN-C responsive element.

Gene expression studies of the cardiac *hH1* sodium channel (*SCN5A*) in human saphenous vein endothelium. A. Scoumanne, J.T. Powell, M. Gosling, N. Carey. Department of Vascular Surgery, Imperial College School of Medicine, Charing Cross Hospital Campus, Fulham Palace Road, London W6 8RF, UK.

Human saphenous vein is the most commonly used conduit for bypass of occlusive arterial disease such as atherosclerosis. However, a third of bypasses fail within the first year due to stenosis and/or thrombosis. Saphenous vein endothelial cell responses to the arterial environment may be an important contributor to these pathological processes. Recently, we described the unexpected presence of a voltage-dependent sodium current in human saphenous vein endothelial cells (HSVECs) (Gosling *et al.*, 1998) resulting from the expression of the cardiac sodium channel *hH1*. Although, we previously showed that *hH1* expression can be regulated by serum, nothing is currently known about the factors regulating its expression and the physiological importance of its presence in the endothelium. We have established real-time quantitative RT-PCR experiments using Taqman to investigate the effects of potential regulatory proteins e.g. smoking metabolites, selected drugs and shear stress on levels of the *hH1* transcript. In addition, we have identified promoter elements for *SCN5A*, which we are assessing for activity in cell lines and *in vivo*, to identify critical promoter elements for expression in vascular endothelium. Primary HSVECs have a limited life span *in vitro*. To obtain a permanent source of HSVECs, cell lines were generated by transfection with simian virus 40 large T-antigen or papillomavirus type 16 E6 and E7 genes. The cell lines were characterised by the presence of the endothelial marker CD31, the ability to bind *Ulex europaeus* lectin I as well as the functional expression of intercellular adhesion molecule-1. Furthermore, HSVEC lines were characterised by the presence of *hH1* transcripts using RT-PCR and functional *hH1* sodium channel by patch-clamp electrophysiology. These constitute a useful resource for the analysis *hH1* regulation in human vascular endothelium. Gosling M, Harley SL, Turner RJ, Carey N, Powell JT. Human saphenous vein endothelial cells express a tetrodotoxin-resistant, voltage-gated sodium channel current. *J. Biol. Chem.* 1998;273:21084-21090.

***DGSI*, a gene deleted in DGS/VCFS, encodes a nuclear phosphoprotein associated with the nuclear matrix. W.**

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DGSI is a gene that maps to human chromosome region 22q11.2 and is deleted in the vast majority of patients with DGS/VCFS. Although *DGSI* is highly conserved in eukaryotic species and is expressed very early in development, computational analysis of the predicted amino acid sequence does not reveal any similarity to known proteins, suggesting that *DGSI* is a member of a novel family of proteins with unknown function. Thus, the characterization of *DGSI* will provide fundamental data that will aid in understanding the function of this protein. In this study, we observed a punctate staining pattern for *DGSI* in nucleoplasm with exclusion from nucleoli by immunofluorescence. The punctate signal was retained in nuclei of cells after *in situ* nuclear matrix preparation, suggesting that *DGSI* is associated with the nuclear matrix. Confocal microscopy was used to produce a series of optical sections through cells at interphase and mitosis. The data show that *DGSI* is excluded from condensed chromosomes during mitosis. In addition, a shift in gel mobility was observed after phosphatase treatment, suggesting that *DGSI* is a phosphoprotein. This result was confirmed by cellular phosphorylation studies. Using the yeast two-hybrid system, we identified a protein, topoisomerase II binding protein (TopBp1), that interacts with *DGSI*. Take together, our studies show that *DGSI* encodes a phosphoprotein associated with the nuclear matrix. Additional experiments are needed to confirm the *DGSI*-TopBp1 interaction and to further characterize the role of *DGSI* in the cell.

Regulatory features of the *Cas-1* genomic organization and evolution associated with oxygen radical metabolism, health and disease. A.K. Black, P.A. Laansoo, N.J. Schisler, B.C. Murphy, S.M. Singh. Zoology, University of Western Ontario, London, Ontario, Canada.

The *Cas-1* gene, encoding the antioxidant enzyme murine catalase, implicated in diverse aspects of aging, cancer, development and alcohol response. In conjunction with SOD and Gpx, this enzyme is responsible for detoxification of O_2^- , and the adverse biological effects associated with it. We have established that this gene is under complex and poorly understood transcriptional and post-transcriptional regulation. Toward a better understanding of its regulatory features, we have assessed the organization, evolution, and associated regulatory features of *Cas-1*. It includes 12 introns, ranging in size from 256bp to 7.5kb. The exon/intron boundaries correspond to the rat and human sequence with species-specific consensus, and the introns contain no apparent regulatory sequences. Two repeats common to more than one *Cas-1* intron appear to be repeated in the introns of a variety of other mouse genes. Such repeats are hypothesized to represent mammalian-specific intronic elements of unknown origin and effect. Further, using sodium bisulphide modifications, the 5' TATA-less promoter was established to be unmethylated in most tissues. These results are compatible with the ubiquitous expression of this gene. Also, the 3' UTR with two specific repeats that actively interact with a set of redox-responsive proteins provides the basis for its post-transcriptional regulation. Finally, we have isolated a 16kb *SalI* 5' 129/SvJ genomic fragment toward generation of *Cas-1* knockouts that are required to fully understand the role of individual genes of the oxygen radical metabolic pathway in health and disease. The observed regulatory features of *Cas-1* argue that this gene could serve as a model for multilevel regulation in response to external challenges.

The Ubiquitin-activating enzyme UBE1 gene produces multiple transcripts in mouse and human. *N. Levy, G. Longepied, M. Mitchell.* Inserm U491, Genetique medicale et Developpement, Faculte de Medecine, Marseille, France.

The ubiquitin-activating enzyme E1 activates ubiquitin and ligation of the activated ubiquitin polypeptide to various proteins targets them for degradation. Ubiquitination is a process whose first step involves the binding of inactive ubiquitin to the ubiquitin-activating enzyme E1. The activated ubiquitin is then transferred to a ubiquitin-conjugating enzyme E2 which transfers the ubiquitin to the protein target, with or without the participation of a ubiquitin ligase E3. There are many different genes for E2 and E3 proteins but only one E1 gene is known and maps to the X chromosome. This suggests that the specificity of ubiquitination is determined largely by the E2 and E3 proteins. We previously identified a testis-specific UBE1 homologue on the mouse Y chromosome, *Ube1y*. *Ube1y* is not present on the human Y chromosome, having been lost during primate evolution. UBE1 may be performing the testis-specific functions of *Ube1y*. To investigate if this role in spermatogenesis is reflected in the transcription of UBE1 and *Ube1x*, we investigated the structure of the transcripts produced by these two genes. One mouse and one human ubiquitous transcripts coding for UBE1 were already known. We investigated the existence of other UBE1 and *Ube1x* transcripts by screening a testis cDNA library and the genbank database and identified multiple mouse and human transcripts, all varying only in their 5'UTR. We have sequenced the genomic region containing these 5'UTR exons in the mouse. We have identified four transcripts in the mouse whose 5'UTR have more than 80% nucleotide identity to four human transcripts. We show that one of these transcripts is expressed predominantly in the testis in human and mouse and may play a role in the degradation of histones during spermiogenesis. The definition of the stage at which this transcript is up-regulated should help elucidate its function. Our results indicate that the selectivity of ubiquitination is controlled in part through the translation of a variety of distinct ubiquitin-activating enzyme E1 transcripts.

Identification of a domain of the FANCD2 protein that is toxic to cells when overexpressed. *D.A. Bruun, J.A. Hejna, R.E. Moses.* Dept Molecular & Medical Gen, Oregon Health Sci Univ, Portland, OR.

The Fanconi anemia complementation group D gene on chromosome 3p25-26 gene has been positionally cloned and identified as FANCD2 (Timmers et al., submitted). The full-length cDNA contains a 4413 bp open reading frame. Like other Fanconi anemia genes, the sequence reveals few identifiable features; however, the FANCD2 cDNA, like FANCA, encodes a functional nuclear localization signal (NLS). This was demonstrated by cloning a 147 bp fragment encoding amino acid residues 1-49 in-frame with enhanced green fluorescence protein (EGFP) in the expression vector pEGFP-N1 (Clontech), resulting in expression of the EGFP fusion protein exclusively in the nucleus. Attempts to demonstrate functional complementation of FA group D cells with full-length FANCD2 cDNA in various expression vectors have been unsuccessful because overexpression of FANCD2 is toxic to cells, as manifested by a failure to obtain stable expressing transfectants. In order to determine which part of the protein was responsible for cellular toxicity, we subcloned fragments of the FANCD2 cDNA in-frame into pEGFP-N1. A 3 kb subclone lacking the C-terminal 471 aa residues was toxic to GM639 fibroblasts within 24 hours after electroporation. Nested exonuclease III deletions of this subclone produced fusion constructs which expressed the respective EGFP fusion proteins in the nucleus without affecting cellular viability. The "toxic" domain of FANCD2 has been localized on subclones which express the respective EGFP fusion proteins in the nucleus. Cell death appears to depend on a region of 300 bp or less. *FANCD2FANCD2FANCAFANCD2*.

Identification and characterization of proteins that interact with midin, the Opitz syndrome gene product. *G. Meroni, S. Cainarca, C. Berti, S. Messali, A. Ballabio.* Telethon Inst Genetics & Med, Milan, Italy.

Opitz syndrome (OS) is a multiple congenital anomaly manifested by abnormal closure of midline structures. The gene responsible for the X-linked form of this disease, MID1, encodes a protein (midin) that contains a RING, two B-boxes, a coiled-coil (the so-called tripartite motif) and an RFP like domain. We found that midin is associated with microtubules throughout the cell cycle. Consistent with the evidence obtained by gel filtration analysis, immunoprecipitation experiments demonstrated the ability of the tripartite motif, and in particular of the coiled-coil domain, to mediate midin homo-interaction. Functional characterization of altered forms of midin, resulting from mutations found in OS patients, revealed that association with microtubules is compromised, while the ability to homodimerize and form multiprotein complexes is retained. We have therefore undertaken experiments aimed at the identification of cellular partners of midin to comprehend the pathways and the molecular mechanisms underlying the OS phenotype. Using both a candidate and a two-hybrid screening approach we have selected a number of clones that we are testing as interactors of midin. The interactions mediated by the N-ter of midin should be maintained in the mutants, while the interactions through the C-ter should be altered in OS patients. Based on this idea we performed two-hybrid screenings using two different baits: the tripartite motif and the C-terminal domain. We are characterizing an N-terminus interactor belonging to a class of proteins known to bind cytoskeletal structures and a C-terminus interactor sharing homologies with a gene involved in development. Moreover, we are characterizing another member of the B-box family of proteins showing a cytoskeleton distribution and that interact with the N-terminal portion of midin. The assessment of these interactions will render possible to draw a hypothesis on the physiological role of midin and its altered function in OS mutant cells.

A mutation in the Cys-loop region of the acetylcholine receptor (AChR) α subunit reduces affinity for acetylcholine (ACh) and compromises gating efficiency. K. Ohno¹, X.-M. Shen¹, A. Tsujino¹, J.M. Brengman¹, M. Gingold², A.G. Engel¹. 1) Neurology, Mayo Clinic, Rochester, MN; 2) Morgantown, WV.

Background: The fast-channel myasthenic syndromes are characterized by fast decaying endplate (EP) currents and abnormally brief channel opening events. We previously reported a fast-channel mutation, eP121L, in the extracellular domain of the AChR ϵ subunit, which decreases affinity for ACh (*Neuron* 1996, 17:157), and another mutation, α V285I, in the M3 transmembrane domain of the AChR α subunit, which affects the channel gating mechanism (*Nat Neurosci* 1999, 2:226). Here we describe a fast-channel mutation in the Cys-loop of the AChR α subunit. **Patient:** A 5-year-old girl with life-threatening myasthenic symptoms since birth has no anti-AChR antibodies and responds partially to anticholinesterase drugs. The number of AChRs per EP, EP ultrastructure, and quantal releases by nerve impulse are normal. The miniature EP potential amplitude is 10% of normal. Patch clamp recordings from EP AChRs reveal normal channel conductance but the opening events are 6-fold briefer than normal. **Mutation analysis:** The patient carries two heteroallelic mutations in the extracellular domain of the AChR α subunit: a missense α V132L in the Cys-loop between Cys128 and Cys142, and a frameshifting null mutation, α 381delC. **Expression studies in HEK cells:** The level of expression of α V132L-AChR is normal, but its open probability and apparent affinity for ACh are markedly reduced. The channel opening episodes of α V132L-AChR are much shorter than of wild-type. Interval-based maximum likelihood analysis of clusters of channel openings elicited by desensitizing concentrations of ACh is consistent with a model in which the diliganded receptor opens in two sequential steps. This model predicts a 14-fold increase in the equilibrium dissociation constant (K_2) of ACh from the diliganded receptor, and an 8-fold decrease in the gating equilibrium constant (q_2). **Conclusions:** The Cys-loop region plays an important role in AChR subunit assembly (*Neuron* 1995, 15:1231). We here show that the Cys-loop region also plays an important role in governing affinity for ACh and efficiency of gating.

The localization of FRG1P in the nucleolus, Cajal bodies and speckles implies a fundamental role in RNA processing. *S. van Koningsbruggen¹, G. Deidda², R.W. Dirks³, A.M. Mommaas⁴, J. Onderwater⁴, G.W. Padberg⁵, R.R. Frants¹, S.M. van der Maarel¹.* 1) Human and clinical genetics, LUMC, Leiden, The Netherlands; 2) Institute of Cell Biology, CNR, Rome, Italy; 3) Department of Molecular Cell Biology, LUMC, Leiden, The Netherlands; 4) Laboratory for Electron Microscopy, LUMC, Leiden, The Netherlands; 5) Department of Neurology, University Hospital Nijmegen, The Netherlands.

The FSHD region gene 1 (FRG1) was cloned as candidate gene for facioscapulohumeral muscular dystrophy (FSHD). Although the sequence is highly conserved between vertebrates and non-vertebrates, the function of the FRG1 protein (FRG1P) is still unknown. To obtain more insight in the biological role of FRG1P, we studied its cellular localization after transient transfections. Colocalization studies demonstrate that FRG1P is localized in the nucleoli, speckles and Cajal bodies after transient transfection. This localization is suggestive for a dynamic interplay between these three components in the nucleus as already suggested by several studies involving snRNP maturation. Immunoelectron microscopy showed that FRG1P is localized in the dense structures of the nucleolus. The functionality of two predicted nuclear localization signals (NLS and BP) in FRG1P was confirmed by studying several deletion constructs. The construct missing the NLS signal showed a diffuse cellular staining, while the construct missing the BP signal, was detected in speckles. Its high evolutionary conservation and its potential role in a fundamental process like RNA processing make FRG1P an attractive target for the position effect variegation mechanism that is hypothesized to underlie FSHD pathogenesis.

Towards understanding the tissue-specific functions of dyskerin, the protein responsible for causing the telomere maintenance disorder (dyskeratosis congenita). *N.S. Heiss¹, A. Kolb¹, R. Salowsky¹, D. Bächner², A. Poustka¹.* 1) Department of Molecular Genome Analysis, German Cancer Research Center (DKFZ), Heidelberg, Germany; 2) Universitätsklinikum Eppendorf, Cell Biochemistry and Clinical Neurobiology, Hamburg, Germany.

X-linked dyskeratosis congenita (DKC) is a progressive multisystem disorder most severely affecting tissues with a high cellular turnover such as skin and blood. Most patients die of bone marrow failure, although the chances of developing various types of cancer are also high. Predominantly missense mutations in the *DKC1* gene are responsible for causing DKC, as well as a more severe allelic variant of the disease, Hoyeraal-Hreidarsson syndrome (HHS). The protein dyskerin is a highly conserved nucleolar protein that functions in rRNA biogenesis and possibly also in the processing of the RNA component (hTR) of the telomerase complex. To unravel the functions of dyskerin together with disease progression, we determined the in situ tissue- and developmental-specific expression pattern of the mouse *Dkc1* transcript. A ubiquitous expression was detected at all stages of development with a notably higher expression confined not only to the rapidly dividing epithelia, but also to specific quiescent neurons of the adult brain. These results indicate that some of the pertinent functions of dyskerin may be more tissue-specific than previously thought and are not limited to rapidly dividing cells. It has been proposed that DKC could be a telomere maintenance disorder, although it remains a challenge to understand the underlying disease mechanism. To generate mouse models of the disease we determined the entire mouse genomic sequence. Further, we are functionally characterizing the promoter regions of both the human and mouse genes. By transfecting a series of *DKC1* promoter constructs expressing a secreted form of alkaline phosphatase, preliminary data indicate that the core promoter activity lies within the first 550 bp 5' of the ATG start codon. To understand the tissue-specific functions of dyskerin we aim to identify the corresponding tissue-specific transcription factors.

The study of a cat eye syndrome candidate gene using model organisms. S.A. Maier¹, D. Bushey¹, L. Podemski¹, P. Dickie², J. Locke¹, H.E. McDermid¹. 1) Biological Sciences, University of Alberta, Edmonton, AB, Canada; 2) HSLAS, University of Alberta, Edmonton, AB, Canada.

Cat eye syndrome (CES) is a human genetic abnormality associated with the duplication of a segment of 22q11.2. It is characterized by defects of the eyes, anus, heart, kidney and face, as well as mental retardation. The CES critical region, which has been sequenced, is thought to contain dose-sensitive genes responsible for the CES phenotype. One promising candidate gene is *CECR1* (Riazi *et al*, 2000). *CECR1* shows significant amino acid identity to several invertebrate growth factors, and is expressed in the outflow tract and atrium of the embryonic human heart, the VII/VIII cranial nerve ganglion, and the notocord, making *CECR1* an excellent candidate gene for CES.

In order to study the function of this gene, we are utilizing two model organisms. Using the genomic sequence of *Drosophila melanogaster*, we have identified six genes with sequence similarity to *CECR1*. These uncharacterized gene products show 38 to 40% amino acid identity over the entire human protein. Each gene has significantly diverged from the others, and preliminary evidence shows expression pattern differences. Although there may be functional redundancy, the amount of divergence may allow subtle phenotypes to emerge upon overexpression or disruption. The completion of the human genome sequence will allow us to determine if *CECR1* paralogues also exist in humans.

We are also producing transgenic mice that overexpress human *CECR1*. Initial low yield of founders suggested that *CECR1* may be detrimental *in utero*. Subsequent lower concentrations of the injected clones have produced three transgenic founders, which are currently being bred. We are also in the process of isolating the mouse homologue for expression studies.

By studying this novel gene family in *Drosophila* and mouse, we hope to shed light on gene function. Overexpression of these genes may elucidate roles in the development of symptoms involved in CES.

Development and analysis of a murine BAC contig syntenic to the Smith-Magenis syndrome common deletion. *J. Yan, C.F. Boerkoel, S.S. Park, J.R. Lupski.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Smith-Magenis syndrome (SMS) is a multiple congenital anomaly syndrome associated with deletion of human 17p11.2. The complex phenotype is thought to result from haploinsufficiency effects of contiguous genes that map within the critical interval. This region is syntenic to the murine chromosome 11 32-34cM region. Using 21 overgo probes made from genes and genetic markers mapped in the murine syntenic region, we constructed a BAC contig of this region by screening the murine RPCI-23 BAC library. Ten BACs (479J7, 82E8, 326M22, 181C17, 345K4, 52O1, 77B23, 40J4, 201K2, 77I12) define the minimal tiling distance across this region. After fingerprinting analysis, these BACs have been submitted to the Baylor Genome Sequencing Center for sequencing. Based on the draft sequences of six BACs, we have confirmed the presence of 7 out of 12 genes formerly mapped in the murine syntenic region and mapped 33 additional ESTs. In addition to formerly mapped genes and these 33 ESTs, we also found four more genes in this region (*Eppb9*, *Pemt2*, *Epsin2*, and *Mapk7*). The latter three have also been found in the human SMS region. The expression pattern of these four genes and some ESTs have been examined by both RT-PCR and Northern blot analysis. We are now extending our contig centromeric and telomeric to the SMS deletion to precisely map the boundaries of the region syntenic to the SMS common deletion. This work will identify all genes in the murine chromosome 11 region that maintains conserved synteny with human 17p11.2; several of which may contribute to phenotypic feature of the SMS.

Variable expressivity, androgen insensitivity and androgen receptor gene mutations. *B. Gottlieb, L. Pinsky, LK. Beitel, M. Trifiro.* Dept Cell Genetics, Lady Davis Inst for Medical Research, Montreal, Quebec, H3T 1E2 Canada.

Variable expressivity is a genetic concept whose mechanism of action is poorly understood. We report on an example of variable expressivity in two families with Androgen Insensitivity Syndrome (AIS). An identical mutation in the Androgen Receptor (AR) gene, Ser 814 Asn, has been identified in two unrelated families. Affected members of one family have a partial AIS (PAIS) phenotype with ambiguous female genitalia while those from the second have a mild AIS (MAIS) phenotype with normal male genitalia. This is one of 25 examples of variable expressivity that have been reported in the Androgen Receptor Gene Mutations Database. Phenotypic variation in most AIS cases has usually been reflected in differences in the kinetic properties of ARs, which has allowed for the correlation of functional properties of ARs with phenotypic expression. However, in this study all ARs exhibited an identical and unique set of kinetic properties. Their ARs were ligand specific, with abnormal kinetics only observed with DHT (high Kd) and with MB (high k). This lack of correlation with their phenotypes could not be explained by the CAG polymorphism in exon 1 of the AR, as they shared the same length (20 CAGs) polyGln tract. Further analysis of the kinetics of the ARs from the MAIS family revealed that while the overall value of Kd with DHT was elevated; there was a wide degree of variability in individual experiments, with the Kd sometimes appearing normal. This suggests a heterogeneous AR population of both mutant and WT ARs, and the possibility that in this family there exists a degree of mosaicism that would account for their virilized phenotype. Similar mosaicism has been observed in 6 other cases of AIS and was found to be somatic in nature, with mutant AR genes undergoing a back mutation to produce WT receptors in some cells. An understanding of the relationship of somatic mutations to variable expressivity, is however, likely to require gene mutation databases that distinguish between germline and somatic mutations. Ultimately, it seems reasonable to expect that somatic mutations and somatic mosaicism is likely to have considerable impact on phenotype expression.

Functional Analysis of the Primary Congenital Glaucoma (PCG) Gene - CYP1B1. *I. Stoilov¹, I. Jansson², J.B. Schenkman², M. Sarfarazi¹.* 1) Molecular Ophthalmic Genetics Laboratory, Department of Surgery, University of Connecticut Health Center, Farmington, CT; 2) Department of Pharmacology, University of Connecticut Health Center, Farmington, CT.

CYP1B1 cDNA was amplified from a retinal cDNA library and 5' modifications were introduced to enhance expression. Plasmids were constructed using pCWori⁺ expression vector. The expression proved to be highly dependent upon the heme precursor d-aminolevulinic acid (ALA), with levels of CYP1B1 increasing about 20-fold, to 920 nmol/l in the presence of up to 2.5 mM ALA. The expressed human Cytochrome P450B1 (CYP1B1) has been purified and antibodies to the protein were raised in chickens. Two PCG mutations G61E (hinge region) and R469W (hem-binding region) were introduced in the expression construct. Difference spectroscopy analysis of bacterial homogenates indicated that the absorption peak at 450nm characteristic for the formation of stable hemoprotein complex is absent or substantially diminished in the mutants compared to the wild-type CYP1B1. Western blot detected the presence of CYP1B1 protein indicating that the mutations most probably do not prevent expression of intact hemoproteins, but alters their stability. Metabolism of progesterone and testosterone by a reconstituted system containing the human CYP1B1 and CYP1A2. Metabolism of progesterone and testosterone by a reconstituted system containing the human CYP1B1 or CYP1A2 was studied. The enzymes are reconstituted by adding NADPH-cytochrome P450 reductase and phospholipid vesicles. NADPH is added to start the monooxygenase reaction. Product identification was done by thin-layer chromatography. Comparative analysis found differences in the metabolic activities of CYP1B1 and CYP1A2. For example 15 α -hydroxylation of testosterone was demonstrated only by CYP1B1. This indicates that although the members of family CYP1 may share some substrates their substrate specificities are not completely overlapping and CYP1B1 deficiency may not be fully compensated by other members of this family. Supported By National Eye Institute (EY-11095); The Mary Jane & Peter DaPuzzo Research Fund of the Glaucoma Foundation and the UCHC-GCRC (M01RR-06192).

Expression of *Macaca fascicularis* Liver Arginase in Erythrocytes. P.S. Kim^{1,2}, R.K. Iyer^{1,2}, K.V. Lu¹, H. Yu², A. Karemi², R.M. Kern², D. Tai¹, J. Miller⁴, S.D. Cederbaum^{2,3}, W.W. Grody^{1,2}. 1) Dept Pathology & Lab Med, UCLA School of Medicine, Los Angeles, CA; 2) Division of Genetics and the Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA; 3) Department of Psychiatry and Pediatrics, UCLA, Los Angeles, CA; 4) Laboratory of Chemical Biology, National Institute of Diabetes and Digestive and Kidney Disease, National Institutes of Health, Bethesda, MD.

Arginase I (AI) has a critical function in mammalian liver as an enzyme responsible for the disposal of nitrogenous wastes from protein catabolism. AI is also expressed in various extrahepatic tissues, and may play a role in providing ornithine for biosynthetic reactions that generate various regulatory molecules such as glutamate, glutamine, GABA, agmatine, polyamines, creatine, proline and nitric oxide. Among these, AI is expressed in red blood cells (RBCs) in humans and certain higher primates. *Macaca fascicularis* was identified as an evolutionary transition species for RBC-AI expression. The *M. fascicularis* AI gene was analyzed to study AI expression in erythrocytes. We found globin enhancer-like elements in the 5' upstream of the AI gene, which provided tantalizing clues for its expression in erythrocytes. Since available erythroleukemia cell lines have no apparent AI activity, erythroid progenitor cells (nRBCs) isolated from cord blood were cultured and used to demonstrate AI expression by immunocytochemical staining using anti-AI antibody. Introduction of EGFP reporter vectors into nRBCs showed that the proximal 1.2 kbp upstream of the AI gene are sufficient for AI expression. Expression of a second arginase isoform, AII, in nRBCs was discovered by cDNA profiling, and this was confirmed by immunoprecipitation with anti-AII antibody. An alternatively spliced AI (AI') variant was observed from erythroid cDNA analysis. The alternative splice acceptor site was found to be located within intron 2, resulting in the insertion of 8 additional amino acids without losing its enzymatic activity. Understanding AI gene regulation in erythrocytes may provide alternative treatment options for arginase deficient patients.

Differential expression and distribution of Dp71 isoforms during PC12 cells differentiation. *C. Montanez¹, B. Cisneros¹, F. Garcia², V. Ceja¹, H. Rosas¹, M. Mustre¹, A. Rendon³, D. Mornet⁴, F. Marquez¹.* 1) Dept. Genetics and Molecular Biol., Cinvestav IPN, Mexico; 2) Dept. Physiology and Neurosciences, Cinvestav IPN, Mexico; 3) Lab. Physiopatol. Retinienne, Strasbourg, France; 4) INSERM UI28, Montpellier, France.

Dystrophin gene product Dp71 is transcribed from a promoter localized between exons 62 and 63 of the gene, the function of this protein is unknown. With the aim of establishing a cell model for studying Dp71 function in nervous system, we adopted PC12 cell line, which has been widely used to investigate the mechanisms underlying neuronal differentiation. In the current study we show by RT-PCR assays that PC12 cells express at least two different Dp71 transcripts generated by alternative splicing of exons 71 and 78. Two Dp71 protein isoforms are observed in PC12 cells by Western blot analysis, Dp71f and Dp71d. The level of Dp71f protein displays an increase along differentiation process while Dp71d remains constant over fifteen days of differentiation. Using immunofluorescence we have examined the localization of Dp71 protein isoforms in PC12 cells. Our results show a localization of Dp71 isoforms to different regions. Dp71f is at cytoplasm being most of it at the cell periphery. During differentiation, PC12 cells showed a similar immunoreactivity to undifferentiated stage. Additionally, neuritic process showed a discontinuous labeling being strong at varicosities and growth cone. Dp71d appears at cytoplasm and at nucleus except nucleolus of undifferentiated cells. At nine days of differentiation, most of the cells show a strong immunoreactivity at nucleus, as well as at cytoplasm and neuritic processes. Our results suggest that Dp71d and Dp71f are functionally distinct in PC12 cells.

Biochemical and genetic analysis of myozenin, a novel human Z line protein of skeletal muscle. *F. Takada, H-Q. Tong, T.G. Thompson, L.M. Kunkel, A.H. Beggs.* Genetics Division, Children's Hospital, Harvard Medical School, Boston, MA.

Myozenin (*MYZN*) is a Z line-specific, α -actinin-binding protein of human skeletal muscle. The *MYZN* gene encodes a unique 32 kDa protein with unknown domain structure and no similarities to proteins in any current databases. Specific interaction of this protein with α -actinin was demonstrated *in vivo* using the yeast two-hybrid system and *in vitro* by co-immunoprecipitation and blot overlay experiments. A yeast two-hybrid screen, using muscle gamma filamin (*FLNC*) as bait, pulled out multiple myozenin clones, suggesting that myozenin may serve as a linker to anchor filamin to α -actinin at the Z lines. Double label indirect immunofluorescence microscopy using anti-myozenin and other sarcomeric protein-specific antisera showed that prior to differentiation, myozenin is diffusely distributed in the peri-nuclear cytoplasm and not associated with sarcomeric α -actinin which is localized to stress fibers and adhesion plaques at the periphery of the myoblasts. During premyofibrillar differentiation, myozenin is segregated away from subcellular compartments containing myosin, however, mature myofibrils contained myozenin localized exclusively at Z lines. Immunofluorescence analysis of muscle from a patient with nemaline myopathy reveals myozenin localized to the nemaline rods. The *MYZN* gene is composed of 6 exons spanning 10 kb on human chromosome 10q22. To determine if mutations of myozenin are responsible for any human congenital myopathies, we have screened the *MYZN* gene in over 75 unrelated patients, including 67 with nemaline myopathy. To date, no convincing pathogenic changes have been identified and we are now expanding the range of phenotypes tested to include patients with limb girdle muscular dystrophy and other neuromuscular disorders.

A unique substitution mutation in the human androgen receptor confers ligand specificity. *S.A. Ghali*², *L.K. Beitel*⁵, *R. Lumbroso*⁵, *L. Pinsky*^{1,2,3,4,5}, *M.A. Trifiro*^{2,3,5}. 1) Biology; 2) Human Genetics; 3) Medicine; 4) Pediatrics; 5) LDI, SMBD-Jewish General Hosp.; McGill Univ., Montreal, Quebec, Canada.

The human androgen receptor (hAR), a member of the large superfamily of nuclear receptors, functions as a transcription factor mediating androgen action and is responsible for normal male sexual development. The hAR is comprised of a C-terminal ligand binding domain (LBD), a central DNA-binding domain (DBD), and a polymorphic N-terminal domain. There is strong evidence to suggest that hAR N/C terminal interactions are crucial to the normal transactivation of androgen-responsive genes. Ligand-dependent transcriptional activation is partially mediated by the intra-LBD activation function 2 (AF-2) domain. This domain is essential in recruiting transcription intermediate factors (TIFs), such as coactivators, which associate directly with steroid receptors, forming a "transcriptionally-ready" pre-initiation complex. Mutations in the hAR LBD can hinder recruitment of these coactivators, disturb normal N/C terminal interactions, and ultimately reduce the transactivational competence of the hAR. We report on a ligand-selective mutant AR containing a single Ser814Asn substitution mutation identified in an individual with partial androgen insensitivity. Using the mammalian two-hybrid system, this mutation was tested for its effect on hAR N/C terminal interactions in the presence and absence of the coactivator TIF2. In the presence of mibolerone, methyltrieneolone and testosterone, N/C terminal interactions for the S814N mutant were ~50% that of the normal hAR, with or without the addition of TIF2. In the presence of 5 α -dihydrotestosterone, N/C terminal interactions further decreased compared to the normal hAR; markedly so in the presence of TIF2. The ligand-specificity of N/C terminal interactions, which is observed even upon addition of TIF2, complements previous reports on the ligand-specific kinetic abnormalities of this mutation. Further analysis of the ligand-specific nature of this mutation will enable us to decipher the intricate molecular events associated with hAR function.

Identification of pancreatitis-associated missense mutations in the cationic trypsinogen gene providing insights into the molecular evolution of the human trypsinogen gene family. *J.M. Chen¹, C. Ferec^{1,2}*. 1) Etablissement Francais du Sang-Bretagne, Brest, France; 2) Centre de Biogenetique, Universite de Bretagne Occidentale and Centre Hospitalier Universitaire, Brest, France.

All of the mammalian trypsinogens in one species (paralogs) are more closely related to each other than to trypsinogens of other species (orthologs), and it has long been suggested that **gene conversion** must have contributed to this process of **concerted evolution**. However, such events, if present, have been largely obliterated by subsequent mutations. Interestingly, the presence of such events has been revealed by the identification of pancreatitis-associated missense mutations in the cationic trypsinogen (T4) gene. To date, two missense mutations, an R122H (R117H) in exon 3 and a N29I (N21I) in exon 2 in the T4 gene, have been identified as causing hereditary pancreatitis, and another variant A16V in exon 2 also reported as a possible predisposing factor to pancreatitis. Of note, we identified a CGC to CAT 2 bp nucleotide substitution resulting in the R122H. The theory that it arose as a **gene conversion** event was strongly supported by the presence of AT at corresponding positions of the highly homologous T6 and T7 genes and a *Chi*-like sequence located 27 nucleotides downstream from the mutation. Similarly, the N29I and A16V in exon 2 could also be explained by such an event: the presence of a possible donor sequence in the functional T8 gene for the N29I mutation and the presence of several possible donor sequences for the A16V mutation among the family members; and the presence of a *Chi*-like sequence and a seven nucleotide inverted repeat split by an asymmetric 21 bp sequence in the 5' and 3' proximity of the A16V and N29I mutations, respectively. The constellation of these observations argues strongly that **gene conversion** is a likely cause of these mutations in the T4 gene and more importantly, by inference, provides concrete evidence for the contribution of **gene conversion** to the molecular evolution of the human trypsinogen family. Additionally, this genetic finding also aids in understanding the pathologic effects of these cationic trypsinogen missense mutations in causing pancreatitis.

On the Fate of Plant or other Foreign Genes upon the Uptake in Food or after Intramuscular Injection. U.

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Food is the most abundant source of foreign DNA intake by all organisms. We have investigated uptake and distribution of food-ingested foreign DNA in model experiments with mice. The DNA of bacteriophage M13 and the cloned gene for green fluorescent protein (GFP) as test genes have been traced from the intestinal contents, via the gut wall, Peyer patches and peripheral white blood cells to spleen and liver. There is evidence that in spleen the recombined test DNA can become covalently linked to mouse pseudogenes. Foreign DNA orally administered to pregnant mice can be transmitted via the placenta to fetuses and newborn animals.

We have now chosen a natural scenario and fed soybean leaves to mice. The distribution of the plant-specific gene for the ribulose-1,5-bisphosphate-carboxylase (rubisco) gene has then been studied in the mouse organism. Starting at 2 h after feeding, the rubisco gene can be recovered almost intact from the contents of the gut. Rubisco gene fragments remain detectable up to 49 h after feeding in the intestine, up to 121 h in the cecum. Thus, plant-associated, naturally fed DNA is more stable in the intestinal tract than naked DNA which had disappeared from the intestinal tract by 18 h after feeding. Rubisco gene-specific PCR products of 337 nucleotides in length have been amplified from spleen and liver DNA. After feeding the cloned GFP gene, GFP-expressing, green fluorescing cells have never been detected in any organ system of the mouse. Hence, there is no evidence for the detectable expression of orally administered genes. Moreover, mice have been fed daily with GFP DNA for eight generations and have been examined for the transgenic state by PCR. The results have been uniformly negative and argue against the germline transfer of orally administered DNA. Upon the intramuscular injection of GFP DNA, GFP gene-specific fragments have been amplified by PCR up to 4 months post injection in DNA from injected muscle, up to 24 h post injection in DNA from liver and blood. GFP fragments can also be retrieved from the intestinal contents. Apparently, the organism eliminates injected foreign DNA via the liver-bile-intestinal route.

Evolution of the human immunoglobulin Vk gene locus. *K. Kawasaki¹, S. Minoshima², K. Shibuya², A. Shintani¹, S. Asakawa¹, H.G. Klobeck³, G. Combriato³, H.G. Zachau³, N. Shimizu^{1,2}.* 1) Dept. of Mol. Biol., Keio Univ. Sch. of Med., Kawasaki, Kanagawa, Japan; 2) Dept. of Mol. Biol., Keio Univ. Sch. of Med., Shinjuku, Tokyo, Japan; 3) Adolf-Butenandt-Inst. für Chem., Physikal. Biochem. und Zellbiol., Ludwig-Maximilians Univ., Munich, Germany.

The complete nucleotide sequence of the entire human immunoglobulin k gene locus of 1,010,706-nt was determined and 131 Vk genes, including 55 newly identified Vk genes were localized. Among these Vk genes, 45 possessed open reading frames (ORFs), while the remaining 86 had obvious defects in the coding regions. The Vk gene locus was composed of inverted duplication of 350-kb DNA segment with an average of 99.1% sequence homology. This duplication has been observed only in humans and not in apes. Among the 118 Vk genes located within two duplication units, 40 possessed ORFs; 17 each reside in both duplication units, while 4 became pseudogenes and 2 were deleted from one of the duplication units. Comparison of the nucleotide sequences of these duplication units of 350 kb revealed that it was divided into 14 distinct homology blocks, each of which has significantly different sequence homology ranging 98.1-99.9%. A homology plot along the duplication units revealed that the sequence homology abruptly changes at the borders of adjacent blocks, suggesting these homology blocks might have been generated by rearrangements rather than different rate of mutation. A homologous recombination between inverted duplication units results in an inversion of the locus. Subsequent meiotic recombination between the original allele and the newly created inversion allele can generate a block with high sequence homology. If this is the mechanism by which the homology blocks of the Vk gene locus were generated, the block with the lowest homology will provide a basis for the accurate estimation of the time of duplication. Based on the sequence difference in the b-globin locus between human and chimpanzee, the inverted duplication took place ~4.85 million years ago, which is immediately after the coalescent time of human and chimpanzee.

The evolutionary origins of the pseudogenes for glucocerebrosidase and metaxin on human chromosome 1q21.

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Gaucher disease, the most common of the lysosomal storage disorders, results from mutations within the gene encoding for the enzyme glucocerebrosidase (GC). The gene for human GC has an nearby pseudogene located 16kb downstream on chromosome 1q21 that shares 96% sequence homology with the functional gene. Metaxin, a convergently transcribed gene located adjacent to the GC pseudogene, also has a highly homologous pseudogene located downstream from the GC gene. Many mutations identified in patients with Gaucher disease are found in the pseudogene sequence, suggesting that the pseudogene contributes to the introduction of mutations within the GC gene through reciprocal crossover or gene conversion. These recombination events have been previously documented to occur between both the GC gene and pseudogene as well as between the metaxin gene and pseudogene. The murine GC and metaxin genes are also located within close proximity on the mouse chromosome 3E3-F1. Sequencing of this region failed to identify nearby murine pseudogenes. Two strategies were used to determine the presence or absence of pseudogenes in other species. After comparing the human and murine GC and metaxin sequences and the human pseudogene sequences, PCR primers were designed to correspond to conserved regions and were used to amplify gene and pseudogene fragments whose size differs. These primers were used to screen DNA from nine different species. Nucleotide sequence alignments demonstrated that the GC protein sequence in all the species shared homology with human glucocerebrosidase. Sequencing analysis also indicated that the GC pseudogene was present in the human and rhesus monkey, but absent from the other species. This was confirmed by Southern analysis using genomic DNA from different species digested with SspI and HindIII. Further investigations using DNA sequencing and Southern blots are being performed to establish the presence or absence of the metaxin gene and its pseudogene in each of the species. Studying the evolutionary origin of the glucocerebrosidase and metaxin pseudogenes may help to elucidate their role in Gaucher disease.

Clinical and molecular correlations in individuals with a Fragile X full mutation. *F. Tassone¹, R.J. Hagerman^{2,3}, A.K. Taylor⁴, P.J. Hagerman¹.* 1) Dept Biochem & Molecular Gen, Univ Colorado Health Sci Ctr, Denver, CO; 2) Child Development Unit and Fragile X Treatment and Research Center, The Children's Hospital, Denver, CO; 3) Department of Pediatrics and JFK Partners, University of Colorado Health Sciences Center, Denver, CO; 4) Kimball Genetics.

Fragile X syndrome is caused by a large expansion of a CGG trinucleotides repeat (>200) in the 5' untranslated region of the FMR1 gene, which is often associated with hypermethylation and downregulation of transcription. FMR1 DNA methylation, transcriptional silencing and the consequent absence of the gene product FMRP result in fragile X syndrome. Although methylation is correlated with the transcriptional silencing of genes, the mechanism by which DNA methylation influences transcription has not been determined.

In a previous study we have reported that unmethylated alleles in the full mutation range are transcriptionally active and in some cases show higher levels of FMR1 messenger RNA relative to normals. In line with this finding, we have measured the FMR1 mRNA expression levels in 50 individuals with a full mutation fully methylated by QF RT-PCR. Surprisingly, we have found that the majority of individuals express FMR1 mRNA, with some subjects having FMR1 mRNA levels in the normal range, despite the presence of complete methylation of the FMR1 promoter region. All individuals show reduced FMRP expression by immunocytochemistry. Methylation pattern studies by sodium bisulfite conversion of genomic DNA are under way in the attempt to identify the nature of the observed variability in FMR1 transcription.

Preliminary analysis demonstrates no significant correlations between molecular measures (mRNA or FMRP) and IQ or Behavioral or Physical Index score. The fact that >50% of males with a fully methylated full mutation have significant levels of FMR1 mRNA suggests that therapy which will enhance translation, and therefore, FMRP levels, would be of great benefit in these individuals.

Characterization of PDZ1, a gene in Xq13.1 encoding a protein with a single PDZ domain expressed in human bone marrow stromal cells. *L.M. King, C.A. Francomano.* Medical Genetics Branch, NHGRI/NIH, Bethesda, MD.

A human bone marrow stromal cell (BMSC) cDNA library was constructed as a resource to identify genes expressed in the development of bone. BMSCs are a population of pluripotent cells capable of differentiating into at least four tissue types: bone, cartilage, hematopoiesis-supportive stroma, and adipocytes. We describe a gene we call PDZ1 isolated from this library. The gene localizes to Xq13.1 at SHGC118320 between DXS983 and DXS995 by radiation hybrid (RH) mapping (Stanford G3 panel, LOD score 12.38) and to Xq13 by fluorescence in situ hybridization (FISH) using a human BAC clone containing the gene. This chromosomal localization makes PDZ1 a candidate gene for the Mendelian disorder, FG Syndrome. The genomic sequence was determined by sequencing the BAC clone. The gene comprises 8 exons and 7 introns that span approximately 3.4 kb. Searches of nucleotide databases suggest that this gene is a member of a multigene family. The 1054 bp cDNA sequence was completed by assembling ESTs from the database, by 5' RACE and was confirmed by RT-PCR. The second exon in the mRNA transcript is alternatively spliced and based on RT-PCR, transcripts containing exon 2 are in low abundance. The 420 bp open reading frame encodes a 140 amino acid protein with a theoretical pI/Mw of 6.65/16 kD. We call the protein PDZ1 because it has a single PDZ domain, which is a domain found in diverse signaling proteins. A 967 bp mouse cDNA sequence was assembled from ESTs in the database. No alternatively spliced transcripts were found based on this assembly technique. The human and mouse cDNA sequences have 83% nucleotide identity and the encoded proteins have 99% amino acid similarity. RH mapping also places the mouse Pdz1 gene on chromosome X (LOD score 12.5) between DXMit229 proximal and DXMit230 distal. Northern analyses using human and mouse multiple tissue blots and species specific cDNA probes shows mRNA expression in various tissues. Further analysis of human and mouse PDZ1/Pdz1 gene expression will help to elucidate the role of this gene in bone biology.

Definition of a "functional R domain" of the cystic fibrosis transmembrane conductance regulator (CFTR). C. Ferec^{1,2}, J.M. Chen². 1) Centre de Biogenetique, Universite de Bretagne Occidentale and Centre Hospitalier Universitaire, Brest, France; 2) Etablissement Francais du Sang-Bretagne, Brest, France.

The R domain of CFTR was originally defined by Riordan et al. (1989) as 241 amino acids from C590 to K830, encoded by exon 13. Such exon/intron boundaries provide a convenient way to define the R domain, but do not necessarily reflect the corresponding functional domain within CFTR. Based upon a comparison of the R domain sequences from 10 species, Dulhanty and Riordan (1994) proposed a two-domain model. RD1, the N-terminal third of the R domain (F587 to E672) is highly conserved; RD2, the large central region of the R domain (W679 to P798) has less rigid structural requirements. This two-domain model was given strong support by the striking observation that mutations clustered in RD1 largely caused a processing defect, while those scattered throughout RD2 showed a normal maturation pattern. And two models of the nucleotide-binding domain (NBD) 1 of CFTR have extended the C-terminus of NBD1 to include part of the N-terminus of the R domain, albeit disagreeing with the length of amino acids included. However, the simple observation that two (S660 and S813) out of the four main phosphorylation sites (S660, S737, S795 and S813) are excluded from RD2 clearly indicates that RD2 still does not satisfy the requirements of a "functional R domain". Nevertheless, knowledge of the CFTR structure and function accumulated over the past decade and re-evaluated in the context of a comprehensive sequence comparison of 14 CFTR homologues enabled us to define such a "functional R domain (**Rf**)", i.e., amino acids C647 to D836. We believe **Rf** is a more realistic representation of the "functional R domain" primarily because it contains all of the important potential consensus phosphorylation sequences. In addition, **Rf** also includes the highly charged motif from E822 to D836, which may be important in keeping the channel closed in a basal or unstimulated state through electrostatic interactions with other CFTR domains. Finally, it includes all of the deletions/insertions in this region. This definition also aids in understanding the effects of missense mutations in this extremely divergent region.

X-linked RING zinc finger, ZNF127-Xp is active during mouse embryonic development. *A.J. Kim, M. Ko, D. Schlessinger.* Laboratory of Genetics, NIH/NIA-IRP, Baltimore, MD.

ZNF127, an autosomal gene that may be associated with Prader-Willi syndrome phenotypes, and is one of a family that encode proteins with both a RING(C3HC4) zinc finger and multiple C3H-zinc finger motifs. The Ring zinc fingers have been implicated both in transcription and in protein-protein interactions. An X-linked human homologue of ZNF17, ZNF127-Xp, had been partially recovered in cDNA form and shown to escape X-inactivation. We have found that the mouse orthologue of ZNF127-Xp is expressed in preimplantation mouse embryos, and isolated a full-length cDNA for the mouse gene from a testis full length-enriched cDNA library, using PCR-based techniques with primer pairs inferred from fragmentary ESTs. Sequencing of the full-length cDNA revealed an insert of 1738 bp, encoding an open reading frame of 515 amino acids. The putative protein contained 4 C3H motifs, 1 C2H2CH motif, and near the carboxy terminus, a RING-finger motif (C3HC4). We have made 3 types of recombinant GST-RING protein in Sf9 cells from constructs with the N-terminal, C-terminal, or complete coding sequences. In cell fractionation experiments the expressed N-terminal peptide was soluble, but the C-terminal and complete RING proteins appeared in the insoluble fraction, and could only be solubilized in complex detergent solutions, suggesting that they may be associated with cytoskeletal or nuclear matrix. In initial studies of expression, Northern blot analysis of mouse and human tissue detected three species of approximately 3.4, 1.9 and 0.8kb. Expression increased during mouse embryonic development and expression patterns different with adult mouse tissues. The highest mRNA expression in adult mouse tissues is in testis, with high levels also present in heart, spleen, brain, and kidney. But particularly in the human, the expression of the 3.4kb mRNA is detected in testis.

Functional Genomics of Single-minded 2 (SIM2) Gene Located on Human Chromosome 21q22.2. *Y. Shimizu¹, A. Yamaki¹, J. Kudoh², S. Minoshima², N. Shimizu².* 1) Department of Medical Genetics, Kyorin University School of Health Sciences, Hachioji, Tokyo; 2) Department of Molecular Biology, Keio University School of Medicine, Shinjuku-ku, Tokyo.

Human SIM2 protein has been postulated to function as an important transcription factor in the development of the central nervous system. To understand the molecular mechanism of SIM2 gene expression, we have analyzed the promoter region of SIM2 gene using SIM2 expressing glioblastoma cell line, T98G. By 5'-RACE method, we found that the major transcription initiation site was at about 1.2kb upstream of the initiation codon. Two series of deletion constructs of the presumed promoter region (between nt-2449 and nt-184) fused with luciferase reporter gene. The results revealed that most significant promoter activity was located in the 60-bp sequence between nt-1385 and nt-1325. Computer analysis predicted the presence of three cis-elements, those for c-myb, E47 and E2F, in this region. A point mutation at the consensus sequence for c-myb reduced substantially the promoter activity, whereas a point mutation at the consensus sequence for E2F increased the promoter activity. The gel shift assay using oligo DNA of various cis-element sequences revealed the presence of protein factor(s) which bind to the cis-element for c-myb. To assess intracellular localization of SIM2 protein, we generated fusion genes of full-length of SIM2 cDNA and its deletion mutants with the green fluorescent protein (GFP). Transient expression study in glioblastoma and HeLa cells revealed that the intact SIM2 protein was accumulated in the nuclei, and that the bHLH-PAS and HST domains were not sufficient for the nuclear translocation of SIM2 protein. These data suggest the translocation signal exists in the carboxy terminal region which is rich in basic amino acids.

The severity of experimental colitis in mice is dependent on the presence of the $\alpha 5$ neuronal nicotinic acetylcholine receptor (nAChR) subunit. A. Orr-Urtreger^{1,2}, M. Kedmi¹, F. Karmeli¹, Y. Yaron^{1,2}, D.

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Cigarette smoking has diverse effects on IBD (Inflammatory Bowel Diseases). It is beneficial in Ulcerative Colitis and deleterious in Crohn's disease. The ingredients and mechanisms involved in the relationship between smoking and IBD remain to be defined. In order to study the possible involvement of the nicotinic receptors in the pathogenesis of IBD we compared the severity of experimental colitis in mice with $\alpha 5$ nAChR subunit deficiency to wild type control mice. Colitis was induced in $\alpha 5$ deficient ($\alpha 5^{-/-}$) and control mice by the addition of 2.5% dextran sodium sulfate (DSS) to the drinking water. Control groups received tap water. Disease activity index was determined by scoring changes in body weight and the presence of gross bleeding in stool. On day seven mice were sacrificed, the colon was dissected and weighted, and sections were obtained for histology and to determine the mucosal MPO activity and PGE_2 generation. Expression analysis, using RT-PCR, demonstrated that the $\alpha 5$ neuronal nAChR subunit is expressed in normal adult mouse colon. DSS induced colitis in the $\alpha 5^{-/-}$ mice was significantly more severe as expressed by the percentage change in body weight, and by disease activity and histological score. Mucosal MPO activity and PGE_2 generation were also higher in DSS treated $\alpha 5^{-/-}$ mice as compared to the respective levels in DSS treated control mice. The augmented severity of DSS induced colitis in $\alpha 5$ deficient mice suggests that this subunit may have an important role in the pathogenesis of experimental colitis. It may further suggest that the role of nicotine in IBD may be mediated via neuronal nicotinic acetylcholine receptors that include the $\alpha 5$ subunit.

Molecular characterization of PRY and TTY2 genes on the human Y chromosome. *L. Stuppia^{1,2}, V. Gatta¹, A. Gaspari¹, R. Grande¹, E. Morizio^{1, 3}, D. Fantasia^{1,3}, R. Mingarelli⁴, G. Calabrese^{1,3}, G. Palka^{1,3}.* 1) Dept di Science Biomediche, Univ G D Annunzio, Chieti, Italy; 2) Istituto di Citomorfologia Umana Normale e Patologica CNR, Chieti, Italy; 3) Servizio di Genetica Medica, Ospedale Civile, Pescara, Italy; 4) CSS-IRCCS Mendel, Roma, Italy.

About 10% of infertile males have Yq microdeletions involving the three loci AZFa, AZFb and AZFc. Several genes have been isolated in these loci, but so far the role played by most of them in male infertility has not been clarified, due to the lack of information about their genomic organization and localization within the Y chromosome. In this study we report the molecular characterization of PRY (PTA-BL related Y) and TTY2 (Testis Transcript Y 2) genes, present with multiple copies within AZFb and on Yp. The cDNA sequences of these genes were used for a database screening and aligned with genomic sequences with high homology to identify exon-intron junctions. It was found that PRY spans 25 kb and is composed by 5 exons, while TTY2 spans 17.8 kb and is composed by 7 exons. Specific primers were derived from the intronic sequences, and PCR analysis was performed on normal controls, 9 infertile patients with different Yq deletions and one female patient with a 45,X,i(Yq) karyotype. Patients with Yq deletions involving entire interval 6 showed loss of exons 3-5 of PRY, while all exons were present in patients with deletions involving AZFc alone. On the other hand, TTY2 exons were present in all infertile patients. In the patient with an i(Yq), PCR showed amplification of all PRY exons, but only of exons 1, 3 and 7 of TTY2. These results suggest that copies in Yp and Yq of these genes are not identical. In fact, patients with large Yq deletions retain only exons 1 and 2 of PRY, suggesting that copies in Yp are partial and likely non functional, while the active copies are mapped in AZFb. Thus, PRY could represent another candidate for male infertility. As to TTY2, it is likely that full-length copies are present only in Yp, since the patient with i(Yq) retained only exons 1, 3 and 7. Thus, this gene would not be involved in male infertility, but could be a candidate for the gonadoblastoma locus on Yp.

Novel gene family encoding putative Ca²⁺-binding proteins with EGF-like modules and a CUB domain. A.

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The epidermal growth factor (EGF)-like domain is one of the most widely distributed protein modules commonly found in cell membrane-bound and extracellular proteins usually involved in extracellular signaling and cell guidance. The common occurrence of the EGF-like domain and its potential involvement in human disease has led to intense study of this module. By comparative sequencing of human chromosome region 11p15.3 and the corresponding region on mouse chromosome 7, we determine the coding sequence and the complete genomic structure of a novel gene which we designated CEGP1 (CUB and EGF-like domain containing protein-1). CEGP1 represents a member of a novel gene family and encodes a protein with 11 EGF-like domains (8 of them with Ca²⁺-binding capability) and a single CUB domain, a widespread module in developmentally regulated proteins. BLASTN homology searches revealed two related genes localized on chromosome 22q (CEGP2) and chromosome 6p (CEGP3). We cloned and sequenced the complete coding sequence of the human and parts of the murine genes. The deduced protein structures of CEGP2 and 3 are quite similar to CEGP1 although the number of EGF-like domains vary. Transcription of the three CEGP family members is very complex due to alternative splicing. Northern blot analysis showed a strong ubiquitous expression of CEGP1 in all fetal and adult human tissues examined. In contrast, CEGP2 showed a very slight expression in adult tissues and expression of CEGP3 could not be detected by Northern blot analysis. Interestingly, expression studies during mouse development revealed a strong expression of CEGP2 in an early stage of mouse embryogenesis (7 dpc) with a sharp decrease in later stages. We are currently investigating the detailed expression pattern of CEGP2 in early mouse development. Functional investigation of the CEGP gene family members and there potential involvement in disease is in progress.

The epsilon-subunit gene of F1FO-ATP synthase on HSA 20q13 shows splice variants in 3' UTR. *C. Gross¹, S. Kussmann¹, A. Hehr¹, K. Koerber¹, H. Miller², J. Peters², I. Hansmann¹, D. Schlote¹.* 1) Institut f. Humangenetik und Medizinische Biologie, Univeritaet Halle, Halle, FRG; 2) Medical Research Council, Harwell, GB.

In order to identify genes on human chromosome region 20q13 with a conserved synteny to the imprinting region 1 (IR1) on mouse chromosome 2, predicting the presence of imprinted genes, a lot of ESTs were mapped into a BAC contig of this region. To isolate full length cDNA we screened Gene Finder pools of different human tissues (RZPD, Berlin) and obtained several cDNA clones for sequencing analysis after rescreening high density filters. So far we could characterize several human cDNA sequences, one of them the human gene of ATP5e with an open reading frame of 51 AS corresponding to a transcript of about 400 bp in Northern blot analysis using a human multiple tissue blot (Clontech) and an exon 3 specific probe. This sequence could be mapped proximal to D20S171 based on our BAC contig spanning about 800 kb around the anchor marker GNAS and EDN3. Analysing several PCR-products amplified out of a marathon ready cDNA library (Clontech) as well as MTP-cDNA clones, sequencing revealed three alternative splice products differing in 3' UTR without affecting protein structure. Furtheron Northern blot analysis as well as in situ hybridisation will give insight into the expression pattern of this new splice variants of the epsilon-subunit gene on human chromosome 20q13. Sequencing of already isolated homologous mouse cDNA clones is in progress and might be relevant for identification of splice variations in mouse as well as to get insight into the possible role of the epsilon-subunit in the mitochondrial F1FO-ATP synthase. This work was supported by grant of the DHGP.

Human Pantothenate Kinase 1 (*PANK1*) Gene: Characterization of the cDNAs, Structural Organization and Mapping of the Locus to Chromosome 10q23.2-23.31. *M.A. Karim*¹, *V.A. Valentine*², *S. Jackowski*^{1,3}. 1) Dept. of Biochemistry, St Jude Children's Res Hosp, Memphis, TN; 2) Dept. of Tumor Cell Biology, St Jude Children's Res Hosp, Memphis, TN; 3) Dept. of Biochemistry, University of Tennessee Medical Center, Memphis, TN.

Pantothenate Kinase (PanK) is the regulatory enzyme in the coenzyme A (CoA) biosynthetic pathway. CoA is the major acyl group carrier in intermediary metabolism and is required for fatty acid mobilization and degradation. We cloned and sequenced three human cDNAs that encode the a, b and g isoforms of PanK1, which have distinct amino termini and are predicted to arise from alternative splicing of transcripts of the same gene. These three protein isoforms share a common carboxy terminus of 40 kDa. Northern blot analysis of human multiple tissues with a common PanK1 cDNA probe shows that PanK1 is abundant in liver and kidney. Human PanK1 a, b and g isoform-specific RT-PCR shows that hPanK1a expression is relatively low in all the tissues except kidney and liver; hPanK1b is abundant in all tissues with the highest level found in the liver followed by kidney; and hPanK1g is also expressed in all tissues. Human PanK1a, PanK1b and PanK1g encode 598, 373 and 400 amino acids, respectively. The hPanK1g transcript includes an Alu exonic sequence which translates into an in-frame insertion of 31 amino acids within the hPanK1b protein. The significance of the insertion on PanK1 biochemical function is unclear. We mapped the novel gene encoding human PanK1, named *PANK1*, by fluorescence in situ hybridization (FISH) to human chromosome 10q23.3-23.31 using a BAC containing human PanK1 genomic DNA as a probe. We also mapped the mouse PanK1 gene by FISH to the conserved syntenic locus of mouse chromosome 19C2-3 using a BAC containing mouse PanK1 genomic DNA as a probe. Determination of the gene structure reveals that the *PANK1* gene spans about 62 kb with 9 exons and 8 introns. Defective PanK gene expression is implicated in metabolic disorders such as diabetes. This research was supported by NIH grant GM45737, Cancer Center (CORE) Support Grant CA 21765, and ALSAC.

Mapping and genomic structure of the gene encoding the p22 light chain of dynactin (DCTN-22). *D.R. Mills¹, S.J. Humphray², C.L. Jackson¹*. 1) Pathology, Rhode Island Hospital, Brown University, Providence, RI; 2) The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, CB10 1SA, Cambridge, UK.

Cytoplasmic dynein is a minus end-directed microtubule-based motor that requires the multiprotein complex dynactin for much of its functional activity. The smallest protein component of the dynactin complex, the p22 light chain (DCTN-22), was recently purified and characterized by other investigators. Immunocytochemistry demonstrated localization of the p22 subunit to the centrosome during interphase, the kinetochores and spindle poles during mitosis, and to the cleavage furrow during cytokinesis. We identified an EST for DCTN-22 and initial YAC-based physical mapping localized this gene to human chromosome region 9p13 between the genes APAH1 and CNTFR. As confirmation, we also mapped this gene to 9p13 by screening the human GeneBridge 3 radiation hybrid panel by PCR. DCTN-22 was most tightly linked to SHGC-33703 with a lod of 16.86. Novel PCR primers were designed from the 3' untranslated region of the human gene and optimized to amplify rat genomic DNA. These primers were used to map DCTN-22 to the telomere of rat chromosome 1p13 (lod>10) by PCR screening of a rat radiation hybrid panel suggesting evidence for conserved homology between these regions of the human and rat genomes. We are currently in the process of mapping additional genes from human 9p13 in the rat to investigate the extent of linkage within this syntenic group. We have initiated single strand conformation polymorphism (SSCP) analysis of human DCTN-22 using a series of nested PCR primers designed from cDNA sequence. The SSCP analysis has identified a silent (C/T) single nucleotide polymorphism (SNP) at nucleotide position 18 of the cDNA coding region. This SNP was confirmed by DNA sequencing of amplified cDNAs. Three of the nested cDNA primer pairs were used to amplify across introns to begin examination of exon/intron boundaries. Two regions of the cDNA appear to have introns too large to amplify by PCR-based methods. We are in the process of sequencing a BAC clone that contains DCTN-22 in order to complete our investigation of the genomic organization of this gene.

Isolation and characterization of a novel gene encoding a protein with UBA and SH3 domains on Human Chromosome 21q22.3; its exclusion for the autosomal recessive deafness locus, DFNB10 . *M. Wattenhofer¹, J. Kudoh², K. Shibuya², S. Minoshima², N. Shimizu², A. Berry³, I. Talior³, B. Bonne-Tamir³, R. Lyle¹, J. Michaud¹, C. Rossier¹, S.E. Antonarakis¹, H.S. Scott^{1,4}*. 1) Medical Genetics, Geneva University, CMU, Geneva, Switzerland; 2) Department of Molecular Biology, Keio, University School of Medicine, Japan; 3) Department of Human Genetics, Sackler School of Medicine, Tel Aviv university, Israel; 4) Genetics and Bioinformatics Group, Walter and Eliza Hall Institute, Royal Parade, Parkville, P.O. Royal Melbourne Hospital, Victoria 3050, Australia.

We have recently refined the critical region for the autosomal recessive deafness locus, DFNB10, to ~1Mb, just telomeric to D21S212, using additional markers (precisely mapped on BAC contigs). In order to identify (i) candidate genes for DFNB10, or (ii) genes that may contribute to some of the Down syndrome phenotypes, we analysed a 74 kb genomic sequence from the DFNB10 critical region and isolated a novel cDNA that maps between TFF1 and D21S49. This gene encodes a putative protein of 661 amino acids and was termed UBASH3A. Alternative splicing results in an isoform of 623 amino acids. UBASH3A is composed of 15 exons distributed over 40-kb. It is expressed in kidney, spleen, lung, PBLs and bone marrow. Both putative proteins contain, in order, an UBA domain (ubiquitin associated, first described in proteins involved in ubiquitination) a SH3 domain, and a previously undefined domain at the C-terminus end. UBA and SH3 domains are thought to be involved in protein-protein interactions. UBASH3A is homologous with a predicted protein (CG13604) in *Drosophila* of unknown function (about 35 % identity at the protein level). Sequence analyses in DFNB10 patients failed to identify pathogenic mutations and therefore this gene was excluded as the cause of DFNB10. By its position, UBASH3A is a candidate for other genetic disorders mapped to 21q22.3, such as one form of bipolar affective disorder.

Epididymal Expression of *SPAM1* in the Mouse and Evidence for a New Category of Spermatogenic Expressed Genes. *P.A. de Leon, Y. He, X. Deng.* Dept Biological Sci, Univ Delaware, Newark, DE.

Introduction: The Gene for the Sperm Adhesion Molecule 1 (PH-20), *SPAM1*, which maps to human 7q31 encodes a GPI-linked protein with hyaluronidase activity and multiple roles in mammalian fertilization. *SPAM1* has been shown to be testis-specific and exclusively haploid expressed. However, a 4-fold increase in the protein (SPAM1) quantity in caudal epididymal sperm compared to caput ones has been observed in mice. **Purpose:** The goal of the study was to determine if SPAM1 is also expressed in the epididymis. **Methods:** Protein extracts from the epididymides of wild-type and germ cell-deficient mice were used for Western blot analysis and hyaluronic acid substrate gel electrophoresis to determine hyaluronidase activity. Steady-state *Spam1* mRNA was analyzed by RT-PCR and by *in situ* hybridization, while confocal microscopy was used in *Spam1* immunolocalization. **Results:** The two common isoforms of *Spam1* seen in sperm were shown to be also present in the caput, corpus, and cauda and were enzymatically active. Qualitative and quantitative variations of expression of *Spam1* were seen, with the highest expression in the corpus. Steady-state *Spam1* mRNA was shown to be localized to the principal cells of the epididymal epithelium where the protein was similarly immunolocalized, predominantly in the apical vesicles. **Conclusions :** The results suggest a mechanism for transport of *Spam1* from the epididymal epithelium to sperm during their maturation in transit and their storage in the cauda. None of the current categories of spermatogenic expressed genes show this dual expression (haploid testicular/diploid epididymal).

Sox transcription factors and the development of the intestine. *W.A. Takash, P. Jay, P. Berta.* Dept Human Molecular Genetics, Inst de Genetique Humaine, Montpellier, Cdx5, Montpellier, France.

Sox genes are members of an evolutionary conserved family encoding transcription factors having homology to the HMG box DNA-binding domain of SRY, the Y-linked testis determining gene. Sox genes show highly restricted spatial and temporal expression profile at different developmental stages. To date, human mutations in SRY, SOX9 and SOX10 cause XY sex reversal, Compomelic Dysplasia and Hirschsprung Waardenburg syndrome respectively. Our laboratory had participated in the cloning and mapping of several Sox genes. In the present study, we have been interested in understanding the function of Sox genes in the physiopathology of the intestine, in both man and mouse. The intestinal epithelium, which is in constant and rapid renewal, provides an attractive system for studying the mechanisms involved in the cell fate determination and differentiation, primarily because of the well-defined architecture of its crypt-villus axis renewal unit. Using different molecular approaches (RT-PCR, Northern, in situ, and whole mounts...), we have detected the expression of several Sox genes in the intestine, including Sox4, Sox5, Sox7, Sox9, Sox13, Sox17 and Sox18. Current investigations involve : 1) FISH analysis, in order to find possible correlations with human diseases, 2) Analysis of alterations of Sox expression patterns in tumorigenesis / carcinogenesis, 3) In vitro experiments towards a functional analysis of these Sox genes in the regulation of intestinal cell functions, namely proliferation and differentiation.

Detailed Analysis of the Pericentromeric 7.1-Mb Region of Human Chromosome 22 Based on the Genomic Sequence. *S. Minoshima, T. Sasaki, K. Kawasaki, S. Asakawa, J. Kudoh, A. Shintani, K. Shibuya, Y. Matsushita, N. Aoki, S. Mitsuyama, K. Hosono, N. Shimizu.* Dept Molecular Biol, Keio Univ Sch Medicine, Tokyo, Japan.

We have finished genomic sequencing of chromosome 22q in collaboration with The Sanger Centre, Oklahoma Univ and Washington Univ as the first human chromosome (*Nature* **402**:489-495, 1999). The complete sequence covers 33.4 Mb and at least 545 genes were found using homology search against sequence database, which include 247 "known-genes", 150 "related-genes" and 148 "predicted-genes". The gene prediction softwares and the analysis of CpG islands predicted additional 200 or more genes. To further evaluate these various classes of genes, we have extensively analyzed 4 separate regions (total 7.13 Mb) : (1) the CES (cat eye syndrome) region (proximal 1.03 Mb), (2) the DGS (DiGeorge syndrome) region (2.5 Mb), (3) BCRL2-IGL-GNAZ region (1.97 Mb), and (4) BCR-IGLL region (1.63 Mb). For the CES region, most genes including NF1-like and ALD-like were considered as pseudogenes except CESK1 (T-complex protein-1 q-like) which is active and the most centromeric gene on 22q. For the BCRL2 region, 16 (pseudo)genes were found including GGT, GGT-related (GGTR), BCRL2 and POM121. For the IGL locus, 36 potentially functional Ig V genes, 83 V pseudogenes, 7 J genes, 7 C genes and 13 non-Ig (pseudo)genes including VPREB and TOP3B were identified. For the IGLL region, 42 genes were identified, including known-genes such as rhabdoid tumor gene (hSNF5/INI1), 2 IGLL genes, BCR, GGT, GGTR, glutathione S-transferase q (GSTT) and 4 D-dopachrome tautomerase (DDCT). GSTT and DDCT were found as a large cluster consisting of 9 GSTT and 4 DDCT (pseudo)genes. Six related-genes and 3 predicted-genes in this region were proven to be active because isolation of cDNAs were successful. Similarly, in the DGS region, cDNAs were isolated for 3 related-genes. In the 22q11.2, LCR22s (Low Copy Repeat on 22q) were found at 7 sites. LCR22s consist of various sequence elements including BCR and GGT, and are strongly associated with the chromosomal breakpoints of various syndromic diseases such as CES, DGS and VCFS.

Analysis and complete genomic sequence of the refined 178 kb Familial Dysautonomia candidate region on

chromosome 9q31. *M. Leyne¹, J. Mull¹, S.P. Gill¹, M.P. Cuajungco¹, C. Karbott¹, B. Johnson¹, C.M. Robbins², I. Makalowska², H.W. Pinkett², C. Maayan³, F.B. Axelrod⁴, A. Blumenfeld³, M. Brownstein², J.F. Gusella¹, S.A.*

Slaugenhaupt¹. 1) Harvard Inst Human Genetics, Massachusetts General Hosp, Boston, MA; 2) National Institute of Health, Bethesda, MD; 3) Hadassah University Hospital, Jerusalem, Israel; 4) New York University Medical Center, New York, NY.

Familial Dysautonomia (FD) is an autosomal recessive disorder that affects the development and survival of sensory, sympathetic and some parasympathetic neurons. To date, the disease has only been described in the Ashkenazi Jewish population where the carrier frequency is 1:30. The FD gene maps to human chromosome 9q31, and haplotype analysis of 441 disease chromosomes initially defined a 471 kb candidate region contained within 58 cosmids and 3 overlapping BAC clones. This region contains 11 candidate genes and the coding sequence of each has been screened using RT-PCR and direct sequencing. This screening did not identify any pathogenic mutations. Because our mutational screening did not identify the FD mutation in the coding sequence of the candidate genes, we set out to sequence the entire 471 kb candidate region using the 3 overlapping BACs spanning the interval. This genomic sequence confirmed that there were no additional genes in the candidate region. In addition, an FD cosmid library was constructed using a patient homozygous for the major haplotype. Sequencing of the FD cosmids allowed us to directly compare the sequence of the candidate interval between affected and control individuals. This sequence comparison identified 2 critical polymorphisms that have narrowed the candidate region from 471 kb to 178 kb. The contiguous 178 kb uninterrupted genomic sequence of this refined candidate interval was determined. To date, 184 DNA sequence differences between the control and FD sequence have been identified. We are currently assessing these DNA changes in FD and control individuals to determine which of these may be the pathogenic FD mutation.

Determination of the genomic sequence of the dystrophin gene. *M. Maheshwari, P.E. Tabor, S. Scherer, R.A. Gibbs.* Department of Molecular & Human Genetics, Baylor college of Medicine, Human Genome sequencing Center, Houston, TX.

Duchenne muscular dystrophy (DMD) is one of the most common X-linked genetic disorders characterized by progressive muscle weakness. It affects about one in 3500 males. The gene responsible for the disease is dystrophin, located at Xp21. It is one of the largest genes with a coding sequence of 14 kb spread over 2,400 kb of genomic DNA and contains 79 exons. DMD is caused primarily by deletion mutations in 60-65% of subjects, while 30-40% of mutations remain uncharacterized. Knowledge of the genomic sequence of the DMD gene will facilitate the search for development of additional methods to detect uncharacterized mutations and carrier detection in females. It may also lead to a mechanistic understanding of DMD mutations and potential insight into understanding the high mutation rate at this locus. Although the exon structure of the DMD gene has been studied, its genomic sequence has not yet been fully characterized. The Human genome project aims to determine the complete nucleotide sequence of the estimated 50000-100000 genes within the human genome. A working draft sequence of 90% of the approximately three billion total bases has been completed, of which the Human Genome Sequencing Center at Baylor College of Medicine has contributed 8.09%. The aim of this study is to determine the sequence extending from exon 44 to 79 of DMD gene. Other regions of DMD are being sequenced by the other centers. To initiate the study, 34 candidate BAC clones were chosen by BLAST searching using sequences of exons and the FPC database produced at Washington University. Candidate BAC clones were screened by exon primer pairs, and primers generated from the BAC end sequences of BAC clones from this region. Positive BACs identified were aligned to determine the extent of overlap between clones and a tiling path of clones were selected for sequencing.

The Integration of Foreign DNA into Mammalian Genomes Can Lead to Alterations in Methylation and Transcription Patterns Remote from the Insertion Site. *K. Mueller, W. Doerfler.* Inst. Genetics, Univ Cologne, Cologne , NRW, Germany.

In earlier work from this laboratory, evidence was presented that in hamster cells transgenic for the DNA of adenovirus type 12 (Ad12) or for the DNA of bacteriophage lambda the patterns of DNA methylation in specific cellular genes or DNA segments had been altered. These alterations were located remote from the site of transgene insertion and were documented by methylation-sensitive restriction endonucleases or by the genomic sequencing technique. We now aimed at selecting a wider scope of cellular DNA segments and genes whose methylation and/or transcription patterns had been altered in transgenic cells as compared to normal cells. The technique of methylation-sensitive representational difference analysis (MS-RDA) was used to isolate differentially methylated DNA fragments. The thus selected DNA segments were then used to verify changes in DNA methylation in the transgenic cells by Southern blot hybridization or by the genomic sequencing technique. The MS-RDA protocol led to the isolation of several cellular DNA segments that were indeed hypermethylated in lambda DNA-transgenic hamster cell lines. In one mouse line transgenic for the DNA of bacteriophage lambda, hypermethylation was observed in the imprinted gene *Igf2r* in a tissue-specific manner. Two mouse lines transgenic for an adenovirus promoter-indicator gene construct showed hypomethylation in the *IL10* and *Igf2r* loci. By applying the suppressive subtractive hybridization technique to cDNA libraries from non-transgenic, Ad12-transformed and lambda DNA-transgenic hamster cells, we were able to clone several cellular genes that showed altered transcription patterns in transformed or in lambda DNA-transgenic hamster cells. In control experiments, no differences in gene expression or DNA methylation patterns were detectable among individual non-transgenic BHK21 cell clones. Many of the DNA segments with altered methylation and/or transcription patterns were identified by their nucleotide sequences. The data presented support the interpretation that the insertion of foreign DNA into an established mammalian genome can lead to marked alterations in cellular DNA methylation and transcription patterns.

Structure of IL-12Rb1 gene and identification of a new polymorphic tetranucleotide repeat. *S. Abdelhak, H. Elloumi, M.R. Barbouche, M. BenFadhel, N. Labben, K. Dellagi.* Immunology Department, Pasteur Institute, Tunis, Tunisia.

The heterodimeric cytokine IL-12 is a powerful inducer of IFN- γ by T and NK cells, thus playing an important role in the control of mycobacteria infection in humans. The IL-12 receptor is composed of two chains b1 and b2 encoded by different genes mapped respectively to chromosomes 19p13.1 and 1p31.3-p31.2. IL-12Rb1 deficiency has been shown to be responsible for severe mycobacterial infections in humans. We have determined the gene structure of IL-12Rb1 by long range PCR amplifications of overlapping fragments and direct sequencing of exon/intron junctions using primers from the cDNA sequence. The gene of IL12Rb1 spans over 26 kilobases of DNA and is organized in at least 16 exons ranging in size from 31 to 238 pb. All splice acceptor and donor sites conform to the AG/GT rule. A tetranucleotide repeat CCAT flanking an ALU element was found in the intron 3 of the gene. Investigation of normal individuals from Tunisian population revealed at least 3 alleles and a heterozygosity frequency of 57% . The intrafamilial genotyping showed a segregation compatible with a mendelian inheritance. This new polymorphism as well as the definition of the genomic structure will facilitate the study of IL-12Rb1 gene mutations and its involvement in the susceptibility to intramacrophagic pathogens.

Variation in the clinical manifestations in Ellis van Creveld may result from differential expression of the EVC gene. *M. Galdzicka¹, P. Damschroder-Williams¹, G. Hirshman¹, R. Long¹, A. Kashani¹, S. Winfield¹, B.M. Martin¹, W.J. Kleijer², E.I. Ginns¹.* 1) Clinical Neuroscience Branch, NIMH/NIH, Bethesda, MD; 2) Clinical Genetics, Erasmus University, Rotterdam, The Netherlands.

Ellis van Creveld syndrome (EvC) is an autosomal recessive disorder with clinical manifestations of dwarfism, polydactyly, ectodermal dysplasia, often congenital heart disease, and occasionally renal failure. We have investigated the molecular basis of EvC in the Old Order Amish in Lancaster County, Pennsylvania, as well as in sporadic cases in other populations. The gene for EvC syndrome has been mapped to human chromosome 4p16.1 and the gene, EVC, has recently been identified. However, the single splice site mutation reported in this gene in the Amish does not readily explain the wide range of observed phenotypes. We have sequenced all 20 exons of the gene in 18 Amish and 7 non-Amish individuals having EvC. One of the Amish EvC individuals does not have the published spliced mutation. Among the non-Amish population, we have identified a heterozygotic novel mutation, 873-874insT in exon 7. We have also used RTPCR to characterize the alternative transcripts of this gene which differ in fibroblasts, lymphoblasts, heart and kidney. We have identified several normal transcripts, among which: one contains 115 bp longer exon 13; one is missing exon 13; and another, only expressed in heart, contains an additional exon, 27 bases long, between exons 2 and 3. In addition to the expression of a variety of transcript isoforms, the EVC mRNA may also undergo RNA editing, providing yet another level by which the EVC gene expression may be regulated and protein diversity elaborated. Our results suggest that the regulation of the EVC gene may be more complex than previously described, and that this may contribute to phenotypic variability of the syndrome.

Genomic organization of ACF7, a candidate for deafness gene *DFNA2*. *T.L. Gong, C.G. Besirli, R.S. Winnicki, M.I. Lomax.* Kresge Hearing Research Institute, Department of Otolaryngology/Head-Neck Surgery, University of Michigan, Ann Arbor, MI.

Mammalian ACF7 (actin cross-linking protein family 7) belongs to the plakin subfamily of structural proteins that cross-link actin cytoskeleton to other structures within the cell. We report here cDNA cloning of a new isoform of human actin cross-linking protein ACF7. In a study on regeneration of auditory hair cells in the chick (Gong et al., Hearing Res. 96, 20-32, 1996), we isolated a partial chick cDNA (KH124). The human orthologue of KH124 maps to chromosome 1p32, in the *DFNA2* region. Although mutations in gene for Connexin31 and potassium channel KCNQ4 in this region have been shown recently to be responsible for deafness in some families, neither of these genes is mutated in an Indonesian family that maps to the *DFNA2* region (Van Hauwe et al., Nat Gen 21, 263, 1999). Thus, our newly identified gene remains a candidate for this Indonesian *DFNA2* family. We cloned a 18-kb human cDNA by screening pituitary and heart cDNA libraries. The C-terminal half of the deduced protein sequence was virtually identical to that of the recently cloned full length ACF7 (named macrophin, trabeculin-a) (Okuda et al., Biochem Biophys Res Comm 264, 568, 1999; Sun et al., J Bio Chem 274, 33522, 1999), containing spectrin repeats, two EF-hands, and a GAS2 domain. In contrast to the three previously cloned isoforms of ACF7 in human and mouse, our newly identified sequence did not contain the sequence for an actin-binding domain (ABD). To determine whether this new sequence is derived from the *ACF7* gene, we isolated and sequenced several human genomic BAC/PAC clones spanning the *ACF7* gene. The human *ACF7* gene consists of at least 104 exons, spans over 200 kb, and gives rise to at least 4 proteins with different N-termini by alternative splicing at the 5' end. The unique N-terminus of our new sequence is encoded by exons internal to the exons for the ABD. The functional significance of these different ACF7 isoforms remains to be determined. (Supported by NIH grants RO1 DC02492, PO1 DC02982).

Gene Structure, Expression and Organization at the *GABRG3* Locus in the Human 15q11-q13 Autism Candidate Region. *M.K. Han, Y.-h. Chen, E.L. Nurmi, J.S. Sutcliffe.* Program in Human Genetics, Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN.

A candidate region for an autism susceptibility locus is localized to an interval on human chromosome 15q11-q13, based on chromosomal duplications, linkage and association studies in families with autistic disorder and broader phenotypes. A cluster of three GABA_A receptor subunit genes (*GABRB3*, *GABRA5* and *GABRG3*) map to this interval and are considered positional and functional candidate genes for involvement in autism. Here we describe the gene structure, organization and expression profile for the $\gamma 3$ subunit gene (*GABRG3*), which is the most distal of the three genes. The *GABRG3* gene is encoded in 10 exons, which span ~400 kb across this region. The gene is expressed at low abundance in adult brain tissues as 4.2-kb or 10.5-kb transcripts, with highest abundance in cerebellum and cortex. *GABRG3* is associated with a CpG island, which is not subject to differential methylation in lymphoblastoid cells. A BAC contig representing the *GABRG3* transcriptional unit was developed and related to emerging phase I human genome sequence and corresponding BAC clones. Analysis of this phase I sequence from the *GABRG3* gene locus indicates the existence of several ESTs representing potential internal genes.

Characterization of the Human NMO-1 gene at 1q43 and genomic organisation of the region. *NMO-1*. S.

*Halford*¹, *J. Bellingham*², *M.S. Freedman*¹, *S.L. Inglis*¹, *S. Poopalasundaram*¹, *R. Foster*², *D.M. Hunt*¹. 1) Department of Molecular Genetics, Institute of Ophthalmology, University College London, London, UK; 2) Department of Integrative & Molecular Neuroscience, Imperial College School of Medicine, Charing Cross Hospital, London, UK.

Light is a potent regulator of many physiological processes in vertebrates. For example, the synchronisation of circadian rhythms to the 24 hr solar cycle and the proper seasonal timing of reproduction are greatly influenced by environmental light cues. In contrast to non-mammalian vertebrates, the photoreceptors mediating non-visual photic processes are ocular in mammals. However, the rod and cone photoreceptors of the retina are not required for the regulation of circadian rhythms or acute suppression of pineal melatonin levels. Therefore, the ocular photoreceptors mediating these non-visual tasks in mammals remain unknown, but it can be reasoned that novel opsin proteins may mediate these responses. As part of an ongoing search to identify novel mammalian photopigments that may be involved in non-visual tasks, recently cloned novel opsin sequences were used to search dbEST. Novel mammalian opsin-1 (NMO-1) was one of the clones identified using this approach. The full-length sequence was compiled from overlapping ESTs and 5' RACE products derived from human retinal cDNA. The predicted protein is 403 amino acids. The gene has two predicted polyadenylation signals that result in transcripts which differ in size by 400bp. Northern blot analysis detects two transcripts of approximately 2.1 and 2.5kb, which are expressed in a wide range of tissues. FISH analysis using a PAC clone and PCR on a monochromosomal somatic cell hybrid panel localised the gene to chromosome 1q43, a region also encompassing the kynurenine monooxygenase (KMO) and choroideremia-like Rab escort protein 2 (CHML) genes. KMO and NMO-1 are transcribed in opposite directions but overlap at the 3' end. CHML, an intronless gene, lies in intron 1 of NMO-1. The intron/exon boundaries of both NMO-1 and KMO have also been deduced. *NMO-1*.

Characterisation of murine CDP-diacylglycerol synthase (CDS1 & CDS2) genes. *S.L Inglis, S. Halford, D.M. Hunt.* Department of Molecular Genetics, Institute of Ophthalmology, University College London, London, UK.

The phototransduction pathway in *Drosophila* is a phosphoinositide-phospholipase C mediated signalling process which results in the signalling molecule phosphatidylinositol-4-5-bisphosphate (PIP₂) being hydrolysed into two second messengers. The regeneration of PIP₂ for subsequent signalling is tightly regulated and involves a number of enzymes. Mutations in many of these genes involved in this pathway give rise to retinal degeneration in flies. As part of an ongoing study to identify mammalian homologues of these genes, two human CDP-diacylglycerol synthase (CDS) genes were identified and clones from retinal cDNA libraries. In *Drosophila*, a retinal specific form of this enzyme is encoded by *eye-cds* and catalyses the formation of diacylglycerol from phosphatidic acid, the rate limiting step in the regeneration of PIP₂ for signalling. Mutations in this gene cause light-induced retinal degeneration in flies. Mammals have been shown to possess two homologues of this gene that are expressed in the retina. In order to determine the full coding sequence and genomic structure of both genes, a full length CDS2 and a partial CDS1 clone were identified in the mouse EST databases. The CDS1 sequence was subsequently completed by 5' RACE. Mouse and human CDS1 and CDS2 sequences show 96% and 93% identity at the amino acid level. Cosmid and PAC genomic clones were obtained by screening the 129 and PAC libraries using fragments from the cDNA clones. From these, we have been able to determine the genomic structure of each gene and to correlate this with the different protein domains of the protein. Finally, a construct for the targeted knockout of CDS2 by homologous recombination has been generated and is presently in use in the production of knockout mice. *Drosophila*₂*eye-cdsl*.

The human *UBE3B* gene encodes a novel E3 ubiquitin ligase with a partial HECT domain. *M.I. Lomax, L. Huang.*
Dept. of Otolaryngology, University of Michigan, Ann Arbor, MI.

Our long term goal is to identify and understand molecular processes involved in the repair and regeneration of the auditory epithelium and in restoration of auditory function after acoustic trauma. We have identified and partially cloned several chick genes whose expression is altered after noise overstimulation. Chicks were exposed to octave band noise (center frequency 1.5 kHz, 118 dB SPL for 6 hr). RNA was isolated from the inner ears of control chicks and from chicks immediately after noise exposure. We identified differentially regulated genes by both differential display and subtractive hybridization, then confirmed differential expression by Northern blot analysis. One differentially regulated gene contained a HECT domain, the signature domain for E3 ubiquitin ligases. This novel E3 ubiquitin ligase, UBE3B, is the second member of this family. The human *UBE3A* gene encodes the first member of this family and is known to be mutated in Angelman Syndrome. In this study, we compared predicted UBE3B HECT domains from chick, human, mouse, and *C. elegans* proteins. The human UBE3B protein deduced from a full length human UBE3B cDNA contains a truncated HECT domain, in contrast to the chick, mouse and worm UBE3B proteins, which have complete HECT domains. Analysis of the intron-exon structure of the human *UBE3B* gene on Chromosome 12q23-25 revealed that the human cDNA arose through alternative splicing of an exon not observed in the UBE3B cDNA sequences from other organisms. Inclusion of this alternatively spliced exon generates a frame shift that leads to premature termination of the UBE3B protein near the beginning of the HECT domain. Since the HECT domain encodes the active site of these E3 ubiquitin ligases, the functional significance of a protein lacking the active site is not clear. Further analysis of human UBE3B transcripts to determine whether or not there are human cDNAs that encode a complete HECT domain are in progress. We are also cloning the mouse Ube3b gene to determine the comparative gene structure. (Supported by NIH grants RO1 DC02492 and PO1 DC02982).

Human transthyretin intronic open reading frames are not independently expressed *in vivo* or part of functional transcripts. *M.L. Soares^{1,2}, M. Centola¹, J. Chae¹, M.J. Saraiva², D.L. Kastner¹.* 1) Arthritis and Rheumatism Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD; 2) Instituto de Biologia Molecular e Celular, Amyloid Unit, Porto, Portugal.

The human transthyretin (TTR) gene encodes a protein composed of four identical subunits with an important role in the plasma transport of thyroid hormone T₄ and retinol. TTR spans about 7.0 kilobases and consists of four exons. Two independent open reading frames (ORFs) with putative regulatory sequences have been described in the first and third introns, but their function - if any - is unknown. We have screened human cDNA libraries to determine if these sequences are transcribed. Transcripts of both ORFs were found in liver, pancreas and brain. Hybridization of the two sequences with multiple tissue northern blots further confirmed these results and revealed transcript sizes of ~1.5 and 2.2 kb for ORF 1, and ~5.2 and ~7.8 kb for ORF 2. Rapid Amplification of cDNA Ends (RACE) was performed to characterize the full-length cDNAs containing each sequence. All products containing the ORFs were continuous in the genomic sequence corresponding to unspliced or partially spliced TTR. No evidence was found for novel transcripts containing productively spliced products of either ORF, or for shorter transcripts using the promoter and polyadenylation signals associated with them. ORF 1 RACE products identified in the liver, pancreas and brain correspond to TTR transcripts in which intron 1 had not been removed; the transcripts containing ORF 2 may represent TTR HN-RNA. Neither ORF is productively expressed as part of a larger transcript, or as an independent polypeptide.

Promoters of the the Human neuronal apoptosis inhibitory protein (NAIP) Gene and the isoform NAIP gene (psi-naip). *M. Xu*¹, *T. Okada*², *N. Miyamoto*¹, *H. Sakai*¹, *S. Hadano*², *R. Korneluk*³, *A. MacKenzie*³, *J.-E. Ikeda*^{1,2}. 1) NeuroGenes (ICORP), Inst Med Sci, NeuroGenes project, ICORP, JST, Kanagawa, Japan; 2) Tokai Univ, Isehara, Kanagawa, Japan; 3) Mol.Genet.Res.Lab., Univ. of Ottawa, Canada.

The neuronal apoptosis inhibitory protein (NAIP) gene has been identified as a candidate gene for spinal muscular atrophy (SMA). The deletion of NAIP is correlated with the clinical severity of the disease. NAIP is duplicated, and one is the deletion isoform gene (psi-naip), which is unknown whether it is transcribable sequence. In this study, we analyzed the 5'-flanking region of the NAIP gene and psi-naip. The 5'-flanking region of 350 bp nucleotides (-765 to -416) was inserted into a luciferase reporter vector, and it revealed apparent promoter activity in monocytic leukemia THP-1 cells but weak activity in neuroblastoma SH-SY5Y and cervical carcinoma C-33A cells. This promoter activity in these human cell lines was appeared by deletion of both of nucleotides from -2218 to -766 and -415 to +294, whereas it was decreased by further deletion from -765 to -483. A 56 bp nucleotides (-483 to -415) in the promoter is essential to promote transcription of NAIP gene. In this region, TATA-like box, an initiator-like sequence, and putative binding sites for GATA-1 and Brn-2 were found. It is noteworthy that the psi-naip promoter region is identical to that of the NAIP gene suggesting that psi-naip is a transcribable unit. Therefore, the identification of transcription factors playing role in the regulation of both gene expression may perceive a molecule pathogenesis of NAIP and psi-naip in SMA.

Genomic organization and expression of the MTHFS gene. *M. Rubini, S. Carturan, I. Bononi, E. Calzolari.*

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The 5-formyl-tetrahydrofolate (5-formyl-THF) is an inhibitor of several folate-dependent enzymes and plays a role in folic acid-dependent one-carbon metabolism. It is synthesized *in vivo* by the Serine-Hydroxymethyl-Transferase (SHMT), an enzyme expressed at steady level, which activity is inhibited by 5-formyl-THF itself. The 5,10-methenyl-tetrahydrofolate-synthetase (MTHFS) is the only enzyme that metabolize the 5-formyl-THF, catalyzing the ATP-dependent conversion to 5,10-methenyl-THF. The combined activities of MTHFS and SHMT constitute a futile cycle that may buffer the 5-formyl-THF concentration. The MTHFS is a 28 kDa polypeptide. Its transcripts are detectable in all tissues, but so far no evidence of modulation of its expression has been reported. The MTHFS gene is located in 15q24.3. It is organized in three exons. The two introns are > 7Kb in length. The gene has no TATA-box, and transcription is initiated at multiple sites at 14-23 nt upstream the translation start codon. Two distinct poly(A) sites have been identified downstream the coding sequence. The expression kinetics of MTHFS enzyme and transcript has been studied in phytohaemagglutinin (PHA)-activated human lymphocytes. Quiescent cells have low transcription while the enzyme level is relatively high. Upon PHA stimulation the 28 kDa protein level drops down, whereas a 20 kDa isoform is expressed. This smaller isoform is due to the use of an alternative transcription initiation site located 12 nt downstream the major translation start codon, within an initiator element consensus sequence. The role and function of this 20 kDa protein is not known. As the activated lymphocytes enter the cell cycle, the 20 kDa isoform is replaced by the 28 kDa MTHFS enzyme. This evidence indicates that the MTHFS expression is modulated during the proliferation process, and this may in turn determine changes in the intracellular 5-formyl-THF level. Regulating the intracellular level of 5-formyl-THF, the MTHFS activity may influence the methyl-THF concentration and the homocysteine remethylation.

***Drosophila* DHFR: Gene expression and methotrexate resistance.** *K.M Al-Batayneh, M. Kuiper, K. Neumann, V.K Walker.* Department of Biology, Queen's University, Kingston, Ontario, Canada.

Dihydrofolate reductase (DHFR) (EC 1.5.1.3) catalyzes the NADPH-dependent reduction of 7,8-dihydrofolate to 5,6,7,8-tetrahydrofolate. DHFR is responsible for maintaining intracellular levels of tetrahydrofolate and its derivatives, which are essential cofactors in the biosynthesis of purines, thymidylate, and several amino acids. Thus, DHFR is a target enzyme for a number of antifolate drugs including the anticancer agent methotrexate (MTX) and is potentially a useful target for novel insecticides.

A computer model of *Drosophila melanogaster* DHFR was constructed by homology modeling to human DHFR. Based on the model, several MTX resistant mutant DHFRs were generated by site-directed mutagenesis. The mutant genes were expressed in *E. coli* to determine the kinetic properties of the recombinant enzymes. Some of these DHFR mutants showed high resistance to MTX, as well as lower affinity to the natural substrate.

Moreover, these mutants as well as a mutant found after selection of *Drosophila* cells on 10^{-4} M MTX have been transfected into *Dhfr*⁻ CHO cell line to assess the ability of these insect genes to confer resistance to MTX in a system that usually depends on gene amplification for the drug resistance phenotype.

Identification of a dominant negative Rieger syndrome mutation in *PITX2*. *I. Saadi*¹, *E.V. Semina*², *D.J. Harris*⁵, *K.P. Murphy*³, *J.C. Murray*^{1,2}, *A.F. Russo*^{1,4}. 1) Genetics Program; 2) Department of Pediatrics; 3) Department of Biochemistry; 4) Department of Physiology and Biophysics, University of Iowa, Iowa City, IA; 5) Children's Mercy Hospital, Kansas City, MO.

Mutations in the *PITX2* bicoid-like homeobox gene cause Rieger syndrome. Rieger syndrome is an autosomal-dominant human disorder characterized by ocular anterior chamber anomalies causing glaucoma in more than 50% of affected individuals, as well as dental hypoplasia, mild craniofacial dysmorphism and umbilical stump abnormalities. *PITX2* has also been implicated in the development of multiple organs and left-right asymmetry in the body plan. The *PITX2* homeodomain has a lysine at position 50, which has been shown to impart the bicoid-type (TAATCC) DNA-binding specificity of other homeodomain proteins through interactions with the last 2 bases of the hexanucleotide element. In a Rieger syndrome patient this lysine is mutated to glutamic acid (K88E). We were intrigued by the marked phenotypic consequences of this mutation. In this study we have examined the functional properties of the mutant *PITX2* K88E protein. The mutant protein had essentially no DNA binding and transactivation activities and, unlike the wildtype protein, the mutant was unable to synergize with another transcription factor, Pit-1. However, when the K88E mutant was coexpressed with wildtype *PITX2*, analogous to the patient genotype, the K88E mutant suppressed the synergism of wildtype *PITX2* with Pit-1 suggesting the formation of nonfunctional complexes between wildtype *PITX2*, K88E mutant, and Pit-1. These results describe the first dominant negative missense mutation in a homeodomain protein and support a model that may partially explain the phenotypic variation within Rieger syndrome and other transcription factor disorders.

A duplication of the glucocerebrosidase pseudogene and a metaxin fusion gene found in patients with Gaucher disease and in normal controls. *N. Tayebi, J.K. Park, V. Madike, E. Sidransky.* Clinical Neuroscience Branch, NIH/NIMH, Bethesda, MD.

The genes for glucocerebrosidase and metaxin, both located on chromosome 1q21, each have a highly homologous pseudogene sequence nearby. We describe a novel recombinant allele consisting of a duplication of the glucocerebrosidase pseudogene and a fusion between the metaxin gene and its pseudogene, resulting from a crossover between metaxin and pseudometaxin in the region downstream of the glucocerebrosidase gene. We also show that certain individuals have a metaxin-pseudometaxin fusion gene without a duplication resulting from the same crossover. DNA from patients with Gaucher disease and normal controls were screened for recombinant alleles by Southern blot analyses prepared with the restriction enzymes SspI and HincII and by direct sequencing. Downstream alterations were identified in eight of the 398 patient alleles studied and in seven of the 200 normal control alleles examined, and were encountered more frequently among patients and controls of African American ancestry. This is the first recognition of a duplicated allele in the glucocerebrosidase gene region and its presence may contribute to genotype-phenotype studies in Gaucher disease.

Tissue and in vitro expression studies of normal human and murine DNAS1L3. *A. Wilber¹, S.A. Abdullah¹, M. Lu¹, M.C. Schneider².* 1) Department of Pediatrics, SIU School of Medicine, Springfield, IL; 2) Division of Research Urology, Brigham and Womens Hospital, Harvard Medical School, Boston, MA.

DNAS1L3 or deoxyribonuclease (DNase) 1 like 3 is one of at least four DNASE 1-like loci in humans. Abnormalities in enzymes of this nuclease family are associated with the predisposition to systemic lupus erythematosus (SLE), an autoimmune disease characterized by a humoral response to nucleosomal antigens. Mutation and dysregulation of this enzyme have been described in polygenic models of SLE. Complete loss of function of DNASE 1 has been found to be associated with a murine lupus phenotype. In a project that aims to understand the role of these nucleases in the predisposition to SLE, we have studied the expression of the DNAS1L3 enzyme in normal human and murine tissues. Previous groups have identified this secreted protein in either macrophages or the nuclei of lymphocytes. Using peptide-specific antisera, we find expression of the murine protein is restricted to liver, spleen, thymus, and bone marrow macrophages. In normal splenocytes, expression of the protein is not inducible by phorbol myristate acetate (PMA), concavalin A, lipopolysaccharide, or activating anti-Fas antibodies. However, in the human Jurkat T-cell line and normal murine macrophages, the expression is highly inducible by PMA. We detect the enzyme in the non-adherent population of human peripheral blood leukocytes. Transfection into COS cells of C-terminal GFP fusions of the enzyme show the enzyme localizes to cell-surface granules and is secreted into the media. N-terminal fusions (lacking a signal peptide) are associated with rapid cell death. We propose potential roles of this enzyme in lymphocyte chromatin fragmentation during apoptosis and macrophage scavenging functions of apoptotic debris.

Imprinting of *PEG3*, the Human Homolog of a Gene Involved in Nurturing Behavior. *S.K. Murphy*¹, *A.A. Wylie*^{1,2}, *R.L. Jirtle*¹. 1) Radiation Oncology and Pathology, Duke University Medical Center, Durham, NC; 2) AstraZeneca Pharmaceuticals, Ltd. Alderley Edge, Cheshire, UK.

The imprinted *Peg3* gene in mice encodes an unusual Krüppel-type zinc finger protein implicated in critical cellular and behavioral functions including growth, apoptosis, and maternal nurturing behavior. We conducted methylation and expression analyses to determine if the human homolog, *PEG3*, on chromosome 19q13.4, is imprinted in humans. The *PEG3* promoter is encompassed within a CpG-rich region that is differentially methylated in human fetal brain, kidney, liver, and pancreas, suggesting regulation of expression in an allele-specific manner. Expression studies using a single nucleotide polymorphism (SNP) within exon 9 revealed that *PEG3* is monoallelically expressed during fetal development. Monoallelic *PEG3* expression was also found postnatally in normal adult brain and ovarian tissues. Using a second SNP, allele-specific expression was demonstrated for two additional isoforms of *PEG3* including a novel isoform. Genotyping of maternal decidua matched to fetal tissues showed that all *PEG3* isoforms are paternally expressed. These results are the first to demonstrate that human *PEG3* is imprinted and that chromosome 19q13.4 contains an imprinted region. The ubiquitous imprinting of *PEG3* coupled with putative roles in regulating cell survival suggest that *PEG3* might function as a cancer susceptibility locus. This study was supported by NIH grants CA25951 and ES08823, DOD grant DAMD17-98-1-8305, Sumitomo Chemical Company, Ltd., and AstraZeneca Pharmaceuticals, Ltd.

Human GRB10 is imprinted and expressed from the paternal and maternal allele in a highly isoform- and tissue-specific fashion. *V.M. Kalscheuer¹, N. Blagitko¹, S. Mergenthaler², U. Schulz¹, H.A. Wollmann³, W. Craig⁴, T.*

Eggermann², H.H. Ropers^{1,5}. 1) Max-Planck-Institute, for Molecular Genetics, Ihnestrasse 73, D-14195 Berlin, Germany; 2) Department of Human Genetics, RWTH Aachen, Germany; 3) Universitaets-Kinderklinik Tuebingen, Germany; 4) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, USA; 5) Department of Human Genetics, University Hospital Nijmegen, The Netherlands.

As part of a systematic screen for novel imprinted genes of human chromosome 7 we have investigated GRB10 which maps to 7p11.2-12. The protein belongs to a small family of adapter proteins, known to interact with a number of receptor tyrosine kinases and signaling molecules. The mouse counterpart was found to be imprinted with expression from the maternal allele only. In mice, maternal duplication of the chromosomal region containing Grb10 results in prenatal growth retardation, whereas paternal duplication causes growth enhancement. Human GRB10 is a candidate for Silver-Russell syndrome, a heterogeneous disorder characterized by intrauterine and postnatal growth retardation, with or without additional dysmorphic features. A subset of patients exhibit maternal uniparental disomy for chromosome 7 (mUPD7) strongly suggesting that genomic imprinting plays a role in the aetiology of the disease. Upon allele-specific transcription analysis involving multiple distinct splice variants in various fetal tissues, we found that human GRB10 in a highly isoform-and tissue-specific manner. In fetal brains, most variants are exclusively transcribed from the paternal allele. Imprinted expression in this tissue is not accompanied by allele-specific methylation of the most 5 CpG island. In skeletal muscle, one GRB10 isoform, gamma 1, is expressed from the maternal allele alone, whereas in numerous other fetal tissues, all GRB10 splice variants are transcribed from both parental alleles. A remarkable finding is paternal-specific expression of GRB10 in the human fetal brain since in the mouse, this gene is exclusively transcribed from the maternal allele. To our knowledge, this is the first example of a gene that is oppositely imprinted in mouse and human.

Expression and methylation studies in mice harbouring a *Snrpn* transgene. *M.J. Smith, D.A. Carter.* School of Biosciences, Cardiff University, Cardiff, Wales, UK.

Imprinted genes are characterised by their differential expression according parent of origin, and are associated with chromosomal regions bearing epigenetic marks that enable discrimination between the maternal and paternal allele. As imprinted genes pass through the germ line, an epigenotype appropriate to the bearers sex has to be established in the gametes. Disruption of the paternally expressed genes in the imprinted domain on human chromosome 15q11-q13 causes Prader-Willi syndrome (PWS). Most cases occur sporadically by *de novo* deletions on the paternally inherited chromosome, or by uniparental disomy of the maternal chromosome 15. Less than 5% of PWS cases are caused by the inheritance of microdeletions that affect the promoter region and exons of the small ribonucleoprotein N gene (*Snrpn*). These deletions delineate an imprinting centre (IC) that influences gene expression and methylation patterns within the entire imprinted domain; they are passed silently through the female germ line, but in the male germ line the female epigenotype is retained. Expression of all the paternal transcripts is consequently silenced in the offspring of male carriers, causing PWS. Mouse chromosome 7C is syntenic to human 15q11-q13 and orthologous genes retain their physical order and imprinted pattern of expression. It is therefore likely that the generation and analysis of mouse models of imprinting will yield important information pertaining to human imprinting disease processes. To this end we have generated lines of mice transgenic for a DNA construct which contains 8 kb of promoter region, exon 1 and the first intron of *Snrpn* linked to a green fluorescent protein reporter. This transgene spans the region equivalent to the human IC, and contains a differentially methylated region that discriminates between maternal and paternal alleles in its native context. Studies of methylation and reporter expression will reveal if sufficient information is present in the transgene to confer imprinting.

Construction of an imprinted status transcript map flanking *MEST/PEG1* at 7q32. K. Yamasaki, S. Hayashida, Y. Tachibana, T. Kishino, N. Niikawa. Human Genetics, Nagasaki Univ., Nagasaki, Japan.

MEST/PEG1, a gene expressed paternally in mesodermal derivatives in early embryonic stages is the first imprinted gene mapped to chromosome 7. Since imprinted genes are clustered in general at a chromosomal region, we speculated that a similar imprinted-gene cluster may exist at 7q32 and that the functions of some such genes may contribute to the phenotype of Silver-Russell syndrome (SRS) including maternal UPD7. Recent linkage analysis revealed that 7q31-q35 region, especially a region near D7S530 and D7S684, is one of the putative autistic disorder (AD) susceptibility loci. In addition, paternal effect in an AD locus on 7q was reported (Ashley-Koch et al., 1999). These findings promoted us to isolate an imprinted gene(s) that implicates SRS or AD. As an initial step toward isolation of imprinted genes at 7q32, we constructed a complete contig of PAC and BAC clones and transcript map spanning the entire ~1-Mb region between D7S530 and D7S649 (Hayashida et al., 2000). Based on the Human Transcript Map, we precisely mapped 47 genes/ESTs and analyzed the imprinting status of several genes/ESTs adjacent to *MEST*. One of them is *COPG2*, coatamer protein complex subunit gamma 2, which overlaps *MEST* 3'UTR. *COPG2* was first reported to be expressed from paternal allele (Blagitko et al., 1999), however, our results revealed biallelic expression in all fetal tissues examined. On the other hand, *CIT1*, coatamer protein complex subunit gamma 2 intron transcript 1, showed paternal expression in fetal tissues. *CPA1*, carboxypeptidase A1, 100 kb centromeric to *MEST* is biparentally expressed. KIAA0265, a cDNA clone with little similarity to any known genes, was mapped several hundred kb centromeric to *MEST* and it also showed biparental expression. So far, we identified only one novel non-coding imprinted transcript, *CIT1*, 30 kb telomeric to *MEST*. These data suggest that 7q32 region is regionally imprinted, but may not make a large imprinted-gene cluster as seen at 11p15.5 and 15q11-q13.

Identification of a novel imprinted domain at human chromosome 14q32. *A.A Wylie^{1,2}, S.K Murphy¹, T.C Orton², R.L Jirtle¹.* 1) Radiation Oncology, Duke University, Durham, NC; 2) Safety Assessment, AstraZeneca, Alderley Edge, Cheshire, UK.

Using a genomics based approach, we have identified two imprinted genes on human chromosome 14q32, which together define a novel imprinting cluster. The first gene, DLK1, belongs to the epidermal growth factor (EGF)-like homeotic family and was named due to its homology with the Drosophila neurogenic proteins Delta and Notch. Delta/Notch signaling is an essential intercellular communication mechanism involved in cell fate decisions. The second gene, GTL2, encodes a non-translated RNA transcript with unknown function. Gene expression analysis in human fetal tissues demonstrated parent of origin dependent, monoallelic expression with DLK1 and GTL2 being expressed exclusively from the paternal and maternal alleles, respectively. Further characterization of the genomic structure of this domain, including methylation analysis, indicated that the spatial organization, differential methylation and reciprocal imprinting of the DLK1/GTL2 domain is very similar to that of the IGF2/H19 domain on chromosome 11. Analysis of GTL2 upstream sequences indicated differential methylation of a putative CTCF binding site. The enhancer-blocking protein, CTCF, has recently been shown to function as a crucial chromatin boundary element involved in directing appropriate expression of IGF2 and H19. These results provide the first characterization of a novel imprinted domain on human chromosome 14 and show imprinting of a human EGF-like homeotic protein with close homology to the Drosophila homeotic protein, Delta.

Identification of potential cis-acting elements regulating expression of the imprinted *SNRPN* gene. *S. Rodriguez-Jato*¹, *D.J. Driscoll*², *T.P. Yang*¹. 1) Department of Biochemistry and Molecular Biology, Center for Mammalian Genetics, University of Florida College of Medicine, Gainesville, FL; 2) Department of Pediatrics, Center for Mammalian Genetics, University of Florida College of Medicine, Gainesville, FL.

The imprinted human *SNRPN* gene is located in the Prader-Willi /Angelman syndrome (PWS/AS) region of human chromosome 15 and is expressed exclusively from the paternally-inherited chromosome. PWS patients with imprinting mutations share a common deleted region located in the 5' region of the *SNRPN* gene that defines the PWS imprinting center (IC) and includes the *SNRPN* promoter region and exon 1. The proximal promoter of *SNRPN* contains a differentially methylated CpG island and displays differential histone acetylation between the paternal and maternal alleles. To identify potential cis-acting regulatory elements that may be involved in imprinted *SNRPN* gene expression and/or IC function, we performed *in vivo* footprint analysis of this region on the paternally and maternally-inherited chromosomes. Lymphoblasts from PWS and AS patients with maternal and paternal uniparental disomy, respectively, were treated with dimethyl sulfate *in vivo* and purified DNA was subjected to ligation-mediated PCR *in vivo* footprinting. To date, we have identified four footprints, all specific to the transcriptionally active paternally-inherited allele, and all clustered within 200 bp spanning the translation initiation site and the immediate 5' flanking region. Only one of the four footprints falls within a series of six boxes in the promoter region that are conserved between the human and mouse genes. These *in vivo* footprinted sites are candidate cis-acting elements that regulate imprinted expression of the *SNRPN* gene and/or participate in PWS-IC function.

Characterization of murine phosphatidylserine-specific phospholipase A1 (*Ps-pla1*): its sequences and

chromosomal location. X. Wen¹, K. Stewart¹, J. Skaug², E. Wei¹, L. Tsui². 1) Dept Experimental Therapeutics, Toronto General Hospital, Toronto, Ontario, Canada; 2) Dept Genetics, The Hospital for Sick Children Research Institute, Toronto, Ontario, Canada.

We have previously generated a mouse transgenic line with an insertional mutation designated *lpd* that demonstrates a phenotype of hypertriglyceridemia and fatty liver. Since the recently identified phosphatidylserine-specific phospholipase A1 (PS-PLA1) demonstrates significant homology to triglyceride lipases, we reasoned that the mouse *Ps-pla1* gene could be the gene disrupted in the *lpd* locus. Using a rat PS-PLA1 cDNA sequence to search EST database, we identified a mouse EST homologue AA839424. Sequencing analysis of AA839424 revealed a putative *Ps-pla1* protein of 456 amino acids with extensive overall structural conservation with human and rat PS-PLA1 and with triglyceride lipases, including a lipase consensus sequences GxSxG, a catalytic triad and eight of the ten conserved cysteine residues that are required for tertiary structure. Mouse *Ps-pla1* carries a phosphatidylserine-binding motif that is absent in all triglyceride lipases. Using a mouse whole genome radiation hybrid (WG-RH) mapping panel (T31), we mapped mouse *Ps-pla1* to chromosome 16 between genetic markers D16Mit194 and D16Mit38 which is 17.1 cM centromeric to the *lpd* locus. Based on chromosome location, we conclude that *Ps-pla1* and *lpd* are distinct genes in lipid metabolism.

A new method for generating modifications in Bacterial artificial chromosomes by homologous recombination in *E. coli*. *M.D. Lalioti, J.K. Heath.* Molecular Cell Biology Group, School of Biosciences, University of Birmingham, UK.

There is a growing need for high throughput techniques to determine gene function and spatial/temporal patterns of expression, as more human genes are predicted or identified. There is also a requirement for the generation of animal models of dominant and gene dosage-dependant genetic diseases. In order to recapitulate the endogenous pattern of expression, it is necessary that the transcriptional and post transcriptional regulatory elements of each gene are preserved. The use of large DNA molecules containing the entire genomic sequence and regulatory elements of a gene is, therefore, the method of choice.

Several methods have been developed in order to modify large clones (BACs/PACs) by homologous recombination in *E. coli*, all of which aimed to introduce insertions or deletions. We developed a new, efficient, and rapid method for introducing point mutations in BACs, in order to study a dominant human disease. We have used the framework of the shuttle vector pKOV, constructed by Link and Church in order to modify *E.coli* genes (J. Bact. 1997). We created a new generation of vectors for mutation of any sequence encoded by BACs in a two step procedure. The new pKOV-Kan contains a temperature-sensitive origin of replication, the chloramphenicol and kanamycin resistance genes for positive and the *sacB* gene for negative selection. A fragment of the gene of interest containing a single point mutation is cloned into the new shuttle vector. This vector and a *recA* expressing plasmid are co-transformed in the BAC cells. In a first recombination event, the "cointegration", the shuttle vector integrates in the BAC. This is followed by a second recombination, the "resolution", which results in BAC which contain either the mutant or the wild type nucleotide. Both steps are selected using resistance or sensitivity conferred by the shuttle vector, and easily confirmed by PCR or southern blot. We have also used this method to engineer an in-frame fusion of our gene of interest and *lacZ*. This allows us to analyse the BAC gene expression in transgenic mice. BACs modified in this manner yield transgenic mice with high efficiency.

Variable X inactivation of human TIMP1: Effect on levels of RNA and role of DNA methylation. *C.L. Anderson, C.J. Brown.* Dept Medical Genetics, Univ British Columbia, Vancouver, Canada.

We have previously demonstrated that the human tissue inhibitor of metalloproteinases-1 (TIMP1) gene shows variable X inactivation being expressed from the inactive X (Xi) in some females but subject to inactivation in other women. As TIMP1 and its target metalloproteinases are involved in many biological processes, women with altered TIMP1 levels may be predisposed to some diseases and metastases. We have used quantitative RPA to establish TIMP1 RNA levels, and observed considerable variation among active X (Xa) chromosomes. Multiple assays have demonstrated 3 origins for this variability: 8% experimental variance; a further 30% reflects cell cycle variation; and lastly a 2x range of TIMP1 expression between different Xas. Diverse expression levels from the Xa precluded analyzing the contribution of the Xi to total TIMP1 RNA in females, so we examined expression in Xi-containing hybrids expressing TIMP1. The Xi levels varied even more widely, with some of the Xi hybrids expressing 5x less TIMP1 than the highest Xa level, which may reflect the presence of residual features associated with X inactivation. To investigate the contribution of methylation, we examined 4 sites at the 5' end of TIMP1 using PCR after methylation-sensitive digestion. TIMP1 is generally unmethylated when expressed, but remained methylated in the lower-expressing Xi hybrids. Since decreased expression in the presence of methylation may reflect a heterogeneous cell population, we analyzed expression in over 100 single cell clones. Although we did detect ongoing instability, with some populations yielding both expressing and non-expressing clones, methylation and low expression (0.2x Xa) were still mutually present in some clones. We conclude that once TIMP1 becomes unmethylated, it behaves like an active copy and therefore could contribute significantly to expression in females. However, the 3 females studied with expression from the Xi have shown methylation of TIMP1. Future studies will analyze more women to establish if TIMP1 becomes unmethylated on the Xi in humans, perhaps preferentially in diseases associated with increased TIMP1.

Cytochrome c oxidase subunit Vb co-localizes with polyglutamine-expanded human androgen receptor. A.

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Spinobulbar muscular atrophy (SBMA) is a late onset motor neuronal disorder caused by the expansion of the polyglutamine (polyGln) tract in exon 1 of the androgen receptor (AR). One way polyGln-expanded proteins are believed to cause neurotoxicity is through abnormal interaction and possible sequestration of critical cellular proteins. Using polyGln-containing fragments of the normal (20Q) and expanded (50Q) hAR as baits, yeast two-hybrid screening of a motor neuron NSC-34 cDNA library led to the identification of COXVb as a new AR-interacting protein. COXVb is a 98 aa nuclear-encoded protein containing a 31 aa signal sequence responsible for mitochondria-targeting. Its interaction with hAR was further substantiated in GST pulldown assays. Mammalian two-hybrid experiments in CV-1 cells showed COXVb to bind to hAR20Q and hAR50Q to the same extent in a hormone-free environment. Addition of androgens increased COXVb-hAR20Q and COXVb-hAR50Q by 4- and 2-fold respectively. Increasing hormone concentrations (up to 10 nM) augmented COXVb-hAR20Q interaction in a dose-dependent fashion, whereas having no effect on COXVb-hAR50Q interaction. Aggregation of COXVb-hAR50Q leading to sequestration of complexes may account for less two-hybrid activity. Co-expression of Hsp70 led to enhanced COXVb-hAR20Q interaction but not of COXVb-hAR50Q. To further document in vivo interactions between COXVb and hAR, COS-1 cells were transfected with either GFP-hAR20Q or GFP-hAR50Q and BFP-COXVb constructs. In several instances, BFP-COXVb was found to co-localize with aggregates of GFP-hAR50Q. This co-localization was restricted to hormone exposure, unique to GFP-hAR50Q co-expression, and only documented within cytoplasmic aggregates. CONCLUSION: Mitochondrial dysfunction may precede classical neuropathological findings and may thus represent an early event in neuronal toxicity. Interaction of COXVb and hAR may provide a mechanism for putative mitochondrial dysfunction in SBMA.

Mapping genes for human mitochondrial ribosomal proteins. *T.W. O'Brien¹, H.-R. Graack², N. Fischel-Ghodsian³, E.B. Mougey⁴, B.A. Maguire⁴, B. Wittmann-Liebold⁵, D.P. Nierlich³, J.E. Sylvester⁴.* 1) Biochemistry and Mol Biol, University of Florida, Gainesville, FL; 2) Institute for Genetics, AG Kress, Free University of Berlin, Germany; 3) UCLA and Cedars-Sinai Medical Center, Los Angeles, CA; 4) Nemours Children's Clinic, Jacksonville, FL; 5) Max-Delbruck-Center for Molecular Medicine, Berlin, Germany.

Mitochondrial DNA encodes tRNAs and rRNAs, but the other genes for the mitochondrial translation system are found in nuclear DNA. Upward of 85 mammalian mitochondrial ribosomal proteins (MRPs) are imported into mitochondria where they assemble into ribosomes that are responsible for translating the 13 mRNAs for essential proteins of the oxidative phosphorylation system. Since mutations in mitochondrial tRNA and rRNA can cause various pathological states, we hypothesize that mutations in MRP genes are also candidates for human disorders. Our approach is to use N-terminal and internal amino acid sequence data obtained from purified bovine MRPs (O'Brien, et al. J Biol Chem. 2000 Apr 5 [epub ahead of print]) to search EST databases. A representative I.M.A.G.E. clone (ATTC) is purchased and used to screen a human lambda genomic library. Chromosome map positions for the MRP genes are ascertained by in situ hybridization (FISH) with genomic sequences and/or by using in silico methods to search Genbank and GeneMap through NCBI. At present, we have over 30 different human MRPs at various stages of characterization. We are currently investigating one MRP as a potential candidate for Russell-Silver Syndrome (RSS), a dwarfism characterized by low birth weight and lateral asymmetry, characteristics that are consistent with reduced mitochondrial function. DNA from RSS patients is being analyzed for possible mutations in MRPs. In addition to studying their clinical relevance, long term characterization of MRP genes should lead to important insights into mammalian evolution, coordinate regulation of nuclear and mitochondrial gene expression, and ribosome function. This work is being done as part of the Mammalian Mitochondrial Ribosomal Consortium and is supported by NIH/NIDCD grant RO1DC04092 and the Nemours Research Program.

Heart-specific splice-variant of human mitochondrial ribosomal protein L5 (MRP-L5). *O. Spirina*¹, *Y. Bykhovskaya*¹, *A.V. Kajava*², *T.W. O'Brien*³, *D.P. Nierlich*¹, *E.B. Mougey*⁴, *J.E. Sylvester*⁴, *H.-R. Graack*⁵, *B. Wittmann-Liebold*⁵, *N. Fischel-Ghodsian*¹. 1) Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA; 2) Center for Molecular Modeling, NIH, Bethesda, MD; 3) University of Florida, Gainesville, FL; 4) Nemours Children's Clinic, Jacksonville, FL; 5) Free University, Max-Delbruck Center, Berlin, Germany.

It has been proposed that splice variants of proteins involved in mitochondrial RNA processing and translation may be involved in the tissue specificity of mitochondrial DNA disease mutations (Mol Genet Metab 65:97, 1998). To identify and characterize the structural components of mitochondrial RNA processing and translation, the Mammalian Mitochondrial Ribosomal Consortium has been formed. The 338 a.a. long MRP-L5 was identified (J Biol Chem 274:36043, 1999), and its cDNA was used to search for tissue specific splice variants. Cyberscreening of the EST databases revealed a single putative splice variant, due to the insertion of an exon consisting of 89 nucleotides prior to the last exon. Screening of multiple cDNA libraries revealed this inserted exon to be present only in heart tissue, in addition to the more abundant MRP-L5 transcript. Sequencing of this region confirmed the EST sequence, and revealed the introduction of a termination codon at the beginning of the last exon. Thus the inserted exon replaces the regular last exon, and creates a new 353 a.a. long protein (MRP-L5V1) with a different C-terminus. Sequence analysis and 3-D modeling reveal similarity between MRP-L5 and threonyl-t-RNA synthetases, and a likely RNA binding site within MRP-L5, with the C-terminus in proximity to the RNA binding site. Sequence analysis of MRP-L5V1 also suggests a likely transmembrane domain at the C-terminus. Thus it is possible that the MRP-L5V1 C-terminus could interfere with RNA binding and may have gained a transmembrane domain. Further studies will be required to elucidate the functional significance of MRP-L5V1.

This work was done as part of the Mammalian Mitochondrial Ribosomal Consortium and is supported by NIH/NIDCD grant RO1DC04092.

Radiation-induced changes in the expression of stress response and DNA repair genes in human and mouse cells.

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Exposure to DNA damaging agents can result in tissue toxicity and lead to diseases, including cancer. We hypothesize that genes exhibiting altered expression within the first few hours after low-dose ionizing radiation (IR) exposure are pivotal in determining cellular responses. We designed custom cDNA microarrays containing genes involved in stress/damage response and DNA repair-related functions. Our microarray system was validated to detect transcript ratio differences of less than 2-fold with strong signal specificity and sensitivity. For these initial studies, we characterized the response of two model systems (human lymphoblastoid cells and whole mouse brain tissue) for relative changes of known genes and to identify novel radiation responsive genes within 7 hrs after 2 Gy IR. Two Coriell Cell Repository human lymphoblastoid cell lines were evaluated using both a brain-specific array and a custom cDNA array. From 57 genes expressed in both cell lines, we found 7 novel and 15 known genes with differential responses between the two cell lines. DNA repair genes showed a slight increase over control samples. The in vivo model used B6C3F1 mouse brain tissue that was collected after whole animal irradiation. Genes important for recombination and repair such as RAD51, ERCC1, MRE11 and RAD52 showed changes in expression patterns that were consistent with the in vitro studies. Several unique gene expression patterns were identified, in addition to those that have been previously described in the literature. These studies have identified genes and pathways that are modulated by IR that will be used to understand the early cellular responses for low-dose irradiation and oxidative stress. [This work was conducted under the auspices of DOE and LLNL under contract W-7405-ENG-48 with support from NIH (ES09117-02) and DOE (KP110202).].

Functional analysis of presenilin 1 in *Drosophila*. *M. Lecourtois, O. Blard, C. Dumanchin, D. Campion, T. Frébourg.* INSERM EMI 9906, IFRMP, Faculté de Médecine et de Pharmacie, 22 Boulevard Gambetta, 76183 Rouen, France.

Alzheimer's disease (AD) is the major neurodegenerative dementia and is characterized by the extracellular deposition of Ab peptide and the presence of intracellular neurofibrillary tangles. Missense mutations of the *PSEN1* gene, located on chromosome 14, account for the majority of autosomal dominant early-onset Alzheimer's disease (ADEOAD) cases. *PSEN1* mutations lead to an increase production of the amyloidogenic Ab 42 peptide, but the mechanisms of this increased production are unknown. To characterize the pathogenic effects of *PSEN1* mutations and the PSEN1 biological pathway *in vivo*, we developed functional analysis of PSEN1 in *Drosophila*. Wild-type or ADEOAD mutant forms of human PSEN1 or its drosophila homolog (DPS) were expressed under the transcriptional control of the endogenous DPS regulatory region. The heterozygote transgenic flies harboring a wild-type and a mutant allele, which mimic the genetic status of ADEOAD patients, exhibited a wing phenotype which was not observed in flies (Wt/-) harboring only one wild-type allele. This phenotype was also observed when we overexpressed truncated forms of PSEN1 binding proteins. These results suggest a dominant negative effect of the PSEN1 mutations which could explain the gain of function of these mutations.

Functional analysis of the gene products of the homologs of the human XNP/ATR-X gene product in

Caenorhabditis elegans and Drosophila melanogaster. F. Usseglio¹, N. Pujol², U. Rothbacher¹, C. Cardoso³, J.

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We have identified single genes highly homologous to the XNP/ATR-X in the genomes of the nematode *Caenorhabditis elegans* and the fly *Drosophila melanogaster*. We have called these new genes *xnp-1* and *dxnp*, respectively. The putative proteins encoded by these two genes are the closest relatives of the human XNP and mouse Xnp proteins known to date. These invertebrate proteins, however, do not possess N-terminal zinc fingers present in the human protein. We are currently undertaking an analysis of the cellular mechanisms in which the two newly identified proteins are involved. In the nematode, we have performed RNAi and *xnp-1* promoter driven eGFP-expression experiments. In *Drosophila*, we have established the *dxnp* transcription pattern during the embryonic development and raised antibodies against the *dxnp* protein to analyse its cytological distribution. We have also taken advantage of the existence of a *Drosophila* line containing a P element in the promoter of *dxnp*. We have induced the excision of the P element and several mutant lines were obtained in which part of the *dxnp* locus is deleted. Lastly, we have undertaken the rescue of the mutant phenotypes with the human XNP/ATR-X protein. These experiments will test the extent to which those two proteins have shared functions at the cellular level.

Towards a mouse model for Smith Magenis Syndrome. *K. Walz, S. Carattini-Rivera, J. Wesley, G. Schuster, J. Yan, C.F. Boerkoel, A. Bradley, J.R. Lupski.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Smith-Magenis syndrome (SMS), caused by del(17)p11.2, represents one of the most frequently observed human microdeletion syndromes. Clinical features include mental retardation, short stature, minor craniofacial anomalies, short fingers, microcornea, developmental defects of the heart and kidneys, and neurobehavioral abnormalities. The majority of patients (>50%) have a common genomic region deleted resulting from an homologous recombination between flanking repeat gene clusters. The complex phenotype features of SMS and the size of the deletion (5-6 Mb) suggests the deletion of several contiguous genes and it is likely that haploinsufficiency of one or more genes in the commonly deleted region underlies this disorder. Although several genes have been identified in the SMS deletion interval, their contribution to this complex phenotype remains speculative. Chromosome 17p11.2 is syntenic to the 32-34 cM region of murine chromosome 11. Our goal is to produce a mouse model using chromosome engineering to construct a deletion of murine chromosome 11 spanning the syntenic region for the human deletion interval in order to define which gene or group of genes is responsible for SMS. COPS3 and ZNF179 are the flanking genes of the human deletion. We targeted both of these genes in mouse ES cells with 2 different vectors carrying the loxP sequence, a coat color marker and two different halves of the Hprt gene for selection of the rearranged chromosomes. The rearrangements were achieved by electroporation with Cre recombinase. Southern blot and FISH were used to analyze the products of the recombination. One ES cell clone gives the deletion and the reciprocal duplication of SMS syntenic region. This clone was injected and the chimeric mice were born. The analysis of the F1 is underway.

The Induced Mutant Resource at The Jackson Laboratory. *S.F. Rockwood, J.J. Sharp, L.E. Mobraaten, M.T. Davisson.* Induced Mutant Resource, The Jackson Laboratory, Bar Harbor, ME.

Transgenic and targeted mutant mice have emerged as the preeminent tool used to undertake the challenge of determining gene function in both normal and disease states. To ensure the greatest possible access to these tools, the Induced Mutant Resource (IMR) at The Jackson Laboratory was created in 1992 to serve as a centralized facility to collect and distribute induced mutant mice to the scientific community. Since its inception, the IMR has accepted over 700 different induced mutant mouse strains, distributing over 100,000 mice a year to the scientific community. Current growth in the IMR collection is approximately 80-100 new strains each year, offering new mouse models in many areas including cancer, diabetes, neurobiology, development and immunology. Each strain accepted into the IMR is vigorously reviewed for biomedical significance and subjected to rederivation procedures that rid them of any pathogens they might carry. Cryopreservation of embryos and sperm derived from these stocks is performed to protect them against accidental loss and genetic contamination. Often, these mutations are placed on defined genetic backgrounds by backcrossing to an inbred strain. An on-line resource is available allowing researchers to retrieve information related to the mutant strains maintained in the IMR. Mutant strain records retrieved from the IMR database include brief phenotype descriptions, strain construction and husbandry information, and a listing of related references. Where appropriate, mutant strain information is linked to entries in other online databases such as the Mouse Genome Database (MGD) and the human Genome Database (GDB). Researchers wishing to have mutant strains considered for inclusion in the IMR collection may submit their strains using the form available at the IMR web site. The IMR database can be accessed at URL: <http://www.jax.org/resources/documents/imr/>. The Induced Mutant Resource is supported by the National Center for Research Resources (RR09781 and RR11081), The National Institute for Arthritis and Inflammatory Disease and The Howard Hughes Medical Institute.

The use of microarrays in the analysis of human skeletal muscle. *J.N. Haslett, D. Sanoudou, H.G.W. Lidov, I.S. Kohane, A.H. Beggs, L.M. Kunkel.* Genetics Division, Children's Hospital, Harvard Medical School, Boston, MA.

The development of cDNA and oligonucleotide arrays has enabled the simultaneous analysis of very large numbers ($>10^3$) genes. Rather than studying single candidate genes for a disease phenotype, expression profiles can be created which represent total gene expression in normal and diseased tissue and specific changes identified. In order to obtain a global expression profile for human skeletal muscle we used Affymetrix oligonucleotide arrays, assaying approximately 7130 genes. This study reports the baseline variation in normal skeletal muscle.

Multiple control experiments were performed using normal human skeletal muscle. Initially identical RNA samples were hybridized to identical chips and compared. 0.3% of genes showed a greater than three-fold difference at their expression level. Two different RNA samples from the same tissue were hybridized on identical chips and compared. 5.2% of genes had an expression level difference higher than three-fold. There was a strong non-linear correlation between expression level and the degree of variation between identical genes. Finally, RNA samples from the skeletal muscle of two different normal individuals were hybridized as before and analyzed. Only 5.7% of genes showed greater than three-fold differences. The differences seen between the samples used for the three paired comparisons are indicative of experimental (i.e. intensity of hybridization signal), inter-microarray, intra-tissue and inter-tissue variation. Data analysis by the Affymetrix GeneChip® software judged approximately 3100 of the 7130 genes studied on each chip to be expressed in the normal human skeletal muscle samples.

In addition to continuing to establish this baseline we are examining muscle specific gene expression profiles in Duchenne muscular dystrophy and Nemaline myopathy patients. We will apply both conventional fold-difference comparisons, as well as automated classification and clustering techniques to identify the genes that are most tightly coupled to those involved in these myopathies. We will also define those genes that most reliably distinguish and characterize these two myopathies.

Identification of a PDZ domain-containing protein scaffold in skeletal and cardiac muscle. *J.L. Siders, C.K. Colton, T.A. Hainsey, J.A. Rafael.* Department of Molecular and Cellular Biochemistry, The Ohio State University College of Medicine Columbus, OH.

The genetic cause for over one hundred described neuromuscular diseases remains unknown. We have identified a family of synapse-associated proteins in skeletal muscle that represent excellent candidates for involvement in this class of disorders. These membrane-associated quanylate kinase proteins have previously been characterized in the brain and have been shown to cluster channels and receptors via their PDZ protein domains. We now show the expression of the three PDZ domain-containing proteins, Dlg, Dlg-3, and CASK in skeletal muscle and heart. CASK and Dlg are expressed at the membrane in cardiac and skeletal muscle. CASK also co-localizes with nuclei in cardiac tissue, providing further evidence for its previously suggested role in modulating gene expression. These results suggest that Dlg, Dlg-3, and CASK may be components of a PDZ domain-containing scaffold similar to the one at central nervous system synapses. Overexpression studies using full-length and PDZ domain-deleted versions of Dlg, Dlg-3, and CASK will delineate the role of these proteins in normal skeletal muscle and heart. Analysis of PDZ domain-containing protein localization in animal models of muscular dystrophy and cardiomyopathy will elucidate the role of these proteins in neuromuscular diseases and heart failure.

Analysis of the transcriptional control of utrophin promoters as a therapeutic strategy for Duchenne muscular dystrophy (DMD). *K.J Perkins, E.A Burton, A.C Potter, K.E Davies.* Human Anatomy and Genetics, University of Oxford, Oxford, UK.

Duchenne muscular dystrophy (DMD) is an X-linked muscle wasting disease caused by the lack of the protein dystrophin. There is no effective treatment for the disorder and patients usually die of cardiac arrest or respiratory failure in their late teens to early twenties. Utrophin is a protein that is closely related in amino acid sequence to dystrophin and its ability to replace dystrophin without toxic side effects is seen to be a major advancement in development of an utrophin-based therapy for DMD individuals. It may be possible to up-regulate endogenous utrophin production in skeletal muscle by a 2 to 3 fold increase in the steady state protein level necessary to prevent dystrophic pathology. One way of achieving this might be through transcriptional up-regulation of the endogenous utrophin gene, analogous to the transcriptional re-activation of γ -globin in β -globinopathies. We have therefore commenced a systematic investigation of the factors responsible for expression of utrophin in order to identify methods of affecting its up-regulation for a promoter-based therapy. Promoter A lies within the CpG island at the 5' end of the gene and contains several transcription start sites, giving rise to the synaptically expressed isoform, whereas promoter B appears to be widely regulated in vitro. EMSA (electrophoretic mobility shift assay) and DNaseI footprinting methods were utilized to delineate functionally important regions of both promoters. Our data demonstrate that the mutagenesis of binding sites of $\text{ap}2$ and $\text{sp}1$ transcription factors within the A promoter results in a significant decrease in promoter activity, supporting the hypothesis that the binding of general transcription factors to this housekeeping promoter is essential for optimal activity. A similar approach has been used to delineate regions of interest in the B promoter and is currently under investigation.

Mutational modelling of missense mutations in Hemophilia A. *P.S. Lai¹, J.H.M. Tan¹, N.O. Cho¹, B. Cheng².* 1) Dept Pediatrics, National Univ Singapore, Singapore; 2) Bioinformatics Centre, National Univ Singapore, Singapore.

Hemophilia A is a common X-linked inherited bleeding disorder caused by molecular defects in the factor VIII (FVIII) gene which codes for coagulation factor VIII protein. Molecular characterisation of FVIII gene has uncovered a wide spectrum of mutations such as nucleotide substitutions, insertions, deletions, splicing errors and gross gene rearrangements. While most of the deleterious mutations result in absence or dysfunctional protein, the effect of missense mutations is unclear. We report the identification of four missense mutations in our patients, G73S, R282C, R2307C and M1934R, the last being novel. Direct sequencing of all exons and flanking introns of FVIII gene did not reveal the presence of a second mutation in all four patients. The missense mutations were analysed for their involvement with common functional motifs using the MOTIFS sequence analysis program. It was found that the mutations in G73S, R282C and M1934R involved the A domains homologous to the copper-binding protein, ceruloplasmin. Homology modelling for secondary structure prediction using Insight II showed that changes in electrostatic interactions in the mutant molecules were likely to result in conformational instability. It is predicted that the metal-ion binding activity of the A domain was adversely affected resulting in severe hemophilia phenotype in G73S while not completely abolishing the functional activity of the protein in R282C and M1934R. The R2307L mutation occurs in the C2 domain of FVIII protein which is homologous to the lipid-binding domain of galactose oxidase. The normal arginine residue in wildtype molecule is highly conserved in both FVIII and FV proteins. This residue may represent an important functional site as molecular modelling shows it being directed towards membrane surface and possibly involved in binding to phosphatidylserine. The change in charges in the mutant molecule is likely to result in structural instability affecting membrane binding, or chemical peptide properties such that the mature protein becomes relatively unstable or rapidly degraded.

Mutational analysis of the skeletal and cardiac muscle specific filamin gene, filamin C (FLNC), in LGMD 1E

patients. *T.G. Thompson*¹, *M.C. Speer*², *H. Webber*¹, *C. Feener*¹, *J.M. Vance*², *L.M. Kunkel*¹. 1) Howard Hughes Medical Institute and Division of Genetics, Children's Hospital, Boston, MA; 2) Department of Medicine, Duke University, Durham, NC.

Gamma filamin, the protein product of the filamin C gene, interacts with members of the sarcoglycan complex, specifically gamma and delta sarcoglycans. There are four sarcoglycan proteins found in skeletal muscle (alpha, beta, gamma and delta) and mutations in any one sarcoglycan gene causes a recessive form of limb-girdle muscular (LGMD 2C-2F). Interestingly, patients and mice with mutations in gamma sarcoglycan, delta sarcoglycan or dystrophin show an increase of gamma filamin at the sarcolemmal membrane indicating a disruption of filamin distribution within skeletal muscle. In addition, families with a dominant form of muscular dystrophy, LGMD 1E, were previously linked to a region on chromosome 7 that may include the FLNC gene. To investigate the possibility of FLNC mutations in these families, we determined the intron/exon borders for FLNC, amplified and sequenced all 48 exons in two affected patients and three control individuals. To date, no apparent disease causing mutations have been found in the LGMD 1E patients, although several single nucleotide polymorphisms (SNP) were found. To eliminate the possibility of undetected mutations or gene deletions, segregation of the newly identified SNPs will be followed through the LGMD 1E families. If recombinational events are found between SNPs in affected individuals, FLNC can be ruled out as a candidate gene for LGMD 1E.

Mammalian operons. *R.D. Nicholls, T.A. Gray.* Dept Genetics, Case Western Reserve Univ, Cleveland, OH.

The accelerating identification of mammalian genes and gene structures from genome projects, and the subsequent characterization of their regulation and functional relationships can be expected to open new avenues of understanding in genome regulation. Here, we have examined evidence leading to the unexpected conclusion that some mammalian loci have features reminiscent of prokaryotic operons. This challenges the decades-old dogma that operons are restricted to prokaryotes, but also reveals additional superimposed levels of regulation more typical of mammalian systems. As defined here, an operon is a single locus that produces transcripts encoding more than one protein and in which the encoded proteins function in a common biological pathway (in contrast, synexpression groups [Niehrs & Pollet, *Nature* **402**:483-487, 1999] have no structural component and are not equivalent to operons). For example, functional L1 repeat elements, the *UOG-GDF1* locus, and the *SNURF-SNRPN* (Prader-Willi syndrome) locus emulate the classical prokaryotic polycistronic mRNA structure, in which the cognate proteins are separately translated from each cistron *in vivo*. Other mammalian operons variously combine alternative splicing, promotion, or translation, or antisense or bidirectional transcription, to diversify and modulate the encoded protein products. Examples include three proteins produced at *GNAS1* (at least two co-localized in secretory granules, and associated with two hormonal disorders), two at *INK4A* (cell cycle regulation, mutated in melanoma), and complex regulation at *MOCS1* and *MOCS2* (both involved in molybdenum cofactor biosynthesis). The evolutionary benefit of an operon is likely the transcriptional and/or translational co-regulation of factors in a common biochemical or physiological pathway. With the completion of human and other vertebrate genome sequences, additional loci will likely be predicted with potential polycistronic or operon structures. Rigorous phylogenetic and *in vivo* mRNA and protein studies of potential mammalian operons will help form a consensus about their evolution, regulation, and physiologic roles in mammalian development.

BIPs and BIPf, newly identified Battenin/Battenim-interactive proteins, may play central roles in the pathogenesis of Batten disease. *N. Zhong^{1,3}, W. Ju¹, D. Moroziewicz¹, A. Jurkewicz¹, K. Wisniewski^{2,3}, W.T. Brown¹.*
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Batten disease (BD), the juvenile form of neuronal ceroid lipofuscinoses (NCLs), is one of the most common pediatric neuronal degenerative disorders in the United States. A gene underlying BD is designated CLN3, which encodes a protein, Battenin, with unknown function. The pathogenic mechanism whereby Battenin, the mutant form of Battenin, results in lipofuscinosis and consequently causes neuronal degeneration is unknown. We hypothesize that there is a common metabolic pathway shared by all NCLs including BD, leading to lipofuscinosis and resulting in pathological neuronal degeneration. Unidentified proteins in this pathway may be functional downstream from, or be regulated by, or along with, Battenin to compose of a protein complex involving in BD as the secondary deficit. We have recently identified two novel Battenin-interactive proteins, BIPs and BIPf, with a yeast two-hybrid system. Our preliminary data revealed that (1) there is an increased interaction of the mutant Battenin with BIPf indicating a genetic deficit of "gain-of-function", (2) BIPs and BIPf interact with each other in addition to Battenin/Battenim, (3) BIPf interacts with mitochondrial ATPase subunit C which has been demonstrated to accumulate in NCLs, including BD, and (4) BIPf interacts strongly with CLN8-encoded protein CLN8p. Our results provided strong evidence supporting our hypothesis, may lead to better understanding of the molecular mechanism underlying BD and shed light on the pathogenesis of NCLs.

Identification of new genes as candidate for imprinting on human chromosome 20q13 segment. *S. Kussmann¹, A. Hehr¹, K. Koerber¹, H. Miller², J. Peters², I. Hansmann¹, D. Schlote¹.* 1) Institut f. Humangenetik und Medizinische Biologie, Univeritaet Halle, Halle, FRG; 2) Medical Research Council, Harwell, GB.

Two mouse models have been generated carrying reciprocal translocations (T1Goe and T30H) which served to define chromosomal segments of parental source effect (Imprinting) on mouse chromosome 2 (MMU2) and shows conserved synteny of gene loci to a human chromosome 20q13 segment. Uniparental disomies for these segments on MMU2 results in different neonatal lethalties with opposite anomalous phenotypes. Using a mouse YAC contig representing one of these imprinting regions (IR1; T(2;8)2Wa and T(2;16)28H), we have assigned several human ESTs, by means of Southern hybridization to PFGE blots of mouse YACs. Based on these results we screened a BAC-library by PCR and isolated a set of 24 BAC clones, covering the syntenic region on human chromosome 20 (HSA20) suspected to carry imprinted genes. Altogether these BACs have been arranged into a preliminary map of approximately 800 kb in length. Sequencing the ends of the BAC inserts results in defining novel STS sites in this segment. Furthermore a total of 13 human ESTs were assigned to the contig and full length cDNA of these ESTs were constructed as well as corresponding mouse cDNA identified. Based on this results we could assign the novel human genes for ATP5e and Hb1 to this segment together with 4 other genes of so far unknown function. Genomic structure and expression analysis will be presented in parallel. These genes are candidates for investigation of monoallelic gene expression and imprinting in mouse and man and will be analysed for their potential association with a given human disease. This work was supported by a grant of the DHGP.

Functional analysis of promoter region polymorphisms. *S.L. Coleman, B. Hoogendoorn, C.A. Guy, S.K. Smith, T. Bowen, A. Halsall, J. Austin, M.C. O'Donovan, P.R. Buckland.* Psychological Medicine, UWCM, CARDIFF, S.GLAM, UK.

The aim of this ongoing project is to identify functional promoter polymorphisms using a screening set of 8 individuals. Denaturing high performance liquid chromatography was used to screen the first 500bp of the 5' flanking region of promoters. To permit T/A cloning an EcoRV site was added to the luciferase reporter gene vector pGL3 to form a T-vector. Insert orientation in the vectors was checked by PCR of E.coli XL1B colonies following transformation. Positive PCR products were mixed in pairs and reanalysed by dHPLC to identify different alleles. Those colonies containing each of the 2 alleles were used for plasmid preparation. The ability of each allele to promote transcription of the luciferase gene is tested transiently in human cell lines HEK293, TE671 and JEG-3. To compensate for number, viability and transfection efficiency of cells a control plasmid SEAP-SV40 was co-transfected. The quantity and ratio of both pGL3 : SEAP DNA and transfection reagent : DNA were optimised for each cell line. Preliminary data shows some promoters have a significant difference in transcription between alleles suggesting that promoter polymorphisms can have functional effects, and, consequently, such polymorphisms should be regarded as a high priority for candidate gene analysis.

Strong association of novel tau promoter polymorphisms with progressive supranuclear palsy. *M. Weiler¹, H.A.R. de Silva^{1,2}, H. Morris², N.W. Wood², A.J. Lees¹.* 1) Reta Lila Weston Institute of Neurological Studies, Royal Free & University College Med. Sch., London W1P 6DB, UK; 2) University Dept. of Clinical Neurology, Institute of Neurology, London, WC1N 3BG, UK.

Purpose: The H1 variant of an extended haplotype spread over 100kb of the *tau* gene is strongly associated with progressive supranuclear palsy (PSP). The functional relevance of this is unclear. In frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP17), splice donor site mutations in *tau* exon 10 cause increased inclusion of exon 10. This results in a pathological increase in four microtubule binding repeat isoforms of tau. Similarities in tau protein deposition between PSP and FTDP17 suggest a similar mechanism in PSP. Since exon 10 mutations are absent in PSP, we analysed the *tau* gene promoter for polymorphisms that could affect tau expression levels. **Methods:** We sequenced a 772bp region that includes 361bp of promoter sequence upstream to the untranslated exon -1 of *tau* in samples homozygous for the *tau* H1 and H2 haplotypes. We identified three single nucleotide polymorphisms (SNP) within the *tau* promoter region that affect *Bsm*AI, *Spe*I and *Alu*I restriction sites, respectively. Analysis of these SNPs in 42 PSP cases (13 pathologically confirmed) and 71 age-matched controls was carried out by PCR amplification of the flanking regions followed by restriction digests. **Results:** We confirmed a previously described SNP, a C®G at position -221 (relative to 5'-end of exon -1) [Ezquerra *et al.* (1999) *Neurosci Lett*], and identified two novel SNPs; an A®G at -44 and G®A at +309 that are in linkage disequilibrium with each other. Restriction analysis of the SNPs in PSP confirm strong association of the allele combination [C(-221)/A(-44)/G(+309)] (PSP=98.8% Contr=78.9%) and homozygotes of this combination (PSP=97.6% Contr=62.0%). Variation at position -221 affects a predicted c-myb binding site whereas the SNP at -44 affects potential AP2 and USF sites. The combination of these two allelic variants could affect *tau* promoter activity and therefore, the expression levels of tau protein, providing a potential functional basis of the well-established haplotype association in PSP.

Hierarchical control of Ectodysplasin-A expression: Involvement of the *Wnt* and EGFR pathways. M.C.

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X-linked anhidrotic ectodermal dysplasia is a human recessive genetic disorder caused by lesions in the EDA gene and characterized by rudimentary teeth, sparse hair, and absent sweat glands. Ectodysplasin-A, the protein encoded by the EDA gene, is a Type II transmembrane protein that forms a collagen triple helix, and may function in cell-cell or cell-matrix interactions at an early point in the development of ectodermal appendages. The EDA gene exhibits a complex hierarchical pattern of regulation, with at least three regions of the promoter responsible for successively more specific levels of control. A first level includes two Sp1 sites near the start site of the gene that drive basal transcription. Upstream regulatory elements of the second and third levels are described here. Level 2, centered at nt-369, contains a binding site for the Lef-1 transcription factor. In co-transfection studies Lef-1 and b-catenin expression plasmids stimulate transcription from the EDA promoter 9-fold in HeLa cells and 7-fold in HaCaT keratinocytes. Mutations in the Lef-1 binding site, gel shift, and supershift experiments confirm that Lef-1 binds specifically to the EDA promoter and is required for full activation of EDA gene expression. Level 3 is a complex enhancer region between nt-673 and nt-550 containing three potential transcription factor-binding sequences within two areas crucial for enhancer activity. Two of the sequences are consensus binding sites for the GATA and Nkx-2 families of transcription factors, which are key regulators of developmental gene expression. The third potential site is a sequence found in the downstream enhancer region of the EGF Receptor gene, to which no known class of proteins binds. Gel shift crosslinking studies implicate four enhancer-binding proteins, each of which has been partially purified by ion exchange and is now being isolated by DNA affinity chromatography. The results indicate that in addition to auxiliary transcription factors, both major pathways classically implicated in epidermal formation (*Wnt* and EGFR) participate in the integration of the EDA gene in skin development.

Identification of a Pea3 responsive element in the *wt1* tumour suppressor gene promoter. *M.T. Discenza*¹, *J. Pelletier*^{1,2}. 1) Biochemistry, McGill University, Montreal, Quebec, Canada; 2) McGill Cancer Center, McGill University, Montreal, Quebec, Canada.

Wilms' tumour 1 (wt1) is a tumour suppressor gene and transcription factor shown to be important for kidney development. Members of the Ets (E26 transformation specific) family of transcription factors are believed to be involved in the regulation of epithelial-mesenchymal interactions during organogenesis, such as during branching morphogenesis in the kidney. The Ets family member polyomavirus enhancer activator 3 (Pea3) and Wt1 have overlapping expression profiles in the mesenchymal compartment of the developing kidney. Analysis of the *wt1* promoter for consensus Pea3 recognition sequences revealed potential binding sites at positions -1019, -864, -696, -425 and +78 with respect to the major start site of transcription. Transfection assays were done using a CAT reporter vector containing the *wt1* promoter together with a Pea3 expression vector. Results from transient transfections in Chinese hamster ovary (CHO) cells revealed that Pea3 is able to transactivate the *wt1* promoter. Progressive 5'-end deletions of the *wt1* promoter were constructed extending to positions -910, -733 and -449. These *wt1* promoter-reporter gene constructs were unresponsive to Pea3 transactivation. Similar experiments were performed with 293 human embryonic kidney cells and comparable results were obtained. This data indicates that the Pea3 responsive element in the *wt1* promoter is located in the vicinity of the consensus Pea3 binding site at position -1019. *In vitro* translation of Pea3 in rabbit reticulocyte lysate yielded a protein of 66 kDa. In an electrophoretic mobility shift assay (EMSA), this Pea3 protein bound to an oligonucleotide containing the Pea3 consensus binding site located at position -1019 of the *wt1* promoter. These preliminary results suggest that Pea3 may be a regulator of *wt1* gene expression during kidney development.

Interaction between SOX10, PAX3 and MITF, three genes altered in Waardenburg syndrome. N. Bondurand¹, V. Pingault¹, D.E. Goerich², N. Lemort¹, E. Sock², C. Le Caignec¹, M. Wegner², M. Goossens¹. 1) Genetique Moleculaire et Physiopathologie, INSERM U468, et Laboratoire de Biochimie et Genetique Moleculaire, AP-HP, Hopital Henri Mondor, 94010 Creteil Cedex, France; 2) Zentrum fur Molekulare Neurobiologie, Universitat Hamburg, Martinistr. 52, 20246 Hamburg, Germany.

Waardenburg syndrome (WS) is an autosomal dominant disorder with an incidence of 1 in 40 000 that manifests with sensorineural deafness and pigmentation defects. It is classified into four types depending on the presence or absence of additional symptoms. WS1 as well as WS3 are due to mutations in the *PAX3* gene whereas some WS2 cases are associated with mutations in the *MITF* gene. The WS4 phenotype can result from mutations in the endothelin-B receptor gene (*EDNRB*), in the gene for its ligand, endothelin-3 (*EDN3*), or in the *SOX10* gene. *PAX3* has been shown to regulate *MITF* gene expression. The recent implication of *SOX10* in WS4 prompted us to test whether this transcription factor, known to cooperate *in vitro* with *PAX3*, is also able to regulate expression from the *MITF* promoter. Here we show that *SOX10*, in synergy with *PAX3*, indeed strongly activates *MITF* expression in transfection assays. Detailed analyses revealed that *PAX3* and *SOX10* interact directly by binding to a proximal region of the *MITF* promoter containing binding sites for both factors. Moreover, *SOX10* or *PAX3* mutant proteins fail to transactivate this promoter, providing further evidence that the two genes act in concert to directly regulate expression of *MITF*. Finally, *in situ* hybridization experiments carried out in the *Dom* mouse, confirmed that *SOX10* dysfunction impairs *Mitf* expression as well as melanocytic development and survival. These experiments, which demonstrate an interaction between three of the genes that are altered in Waardenburg syndrome, could explain the auditory-pigmentary symptoms of this disease.

Transcriptional regulation of the *GPC3* gene in embryonal tumors. G. Boily¹, Z. Saikali¹, S. Ouellet², R. Drouin², D. Sinnett¹. 1) Service d'Héματο-Oncologie, Centre de Recherche, Hôpital Sainte-Justine, Université de Montréal, Montréal, Canada; 2) Unité de Recherche en Génétique Humaine et Moléculaire, Pavillon Saint-François-d'Assise, Université Laval, Québec, Canada.

We have previously shown that the *Glypican 3 (GPC3)* gene was expressed in embryonal tumors, including neuroblastoma (NB), Wilms' tumor (WT), but not medulloblastoma. *GPC3* is an X-linked gene that is almost exclusively expressed in embryonal tissues. We propose that *GPC3* could play a role in the etiology of embryonal tumors. To understand the mechanisms regulating the expression of this gene, we performed DNA methylation studies and *in vivo* footprinting analysis in the promoter region. Southern Blot and PCR-derived methylation assays were used to assess the methylation status of the *GPC3* promoter in genomic DNA from both normal and tumoral cells. In normal cells, we found that the methylation patterns did not correlate with the expression but rather with the gender (absence of methylation in male, partial methylation in female). Except for a few cases, similar methylation patterns were observed in tumor cells. These results suggest that DNA methylation of the promoter region plays little or no role in the transcriptional repression of the *GPC3* gene and that the partial methylation observed in females is probably linked to the inactive X chromosome. To determine whether transcription factors (TF) could be involved in the control of the expression of *GPC3*, we performed *in vivo* footprinting analysis in order to map putative TF binding sites in the promoter region. Two NB cell lines, SJNB-7 (expressing *GPC3*) and SK-N-FI (not expressing *GPC3*), both derived from males, were treated with dimethyl sulfate (DMS) and UV light (UVC). The presence of protected or hypersensitive regions was then revealed by ligation-mediated PCR (LM-PCR). Several footprints were found and many of them were shared by both cell lines. However, some footprints were specific to the expressing cell line (SJNB-7), but not the opposite. These results suggest that the transcriptional activity of *GPC3* requires the presence of a number of specific TFs at the promoter level.

Transcriptional regulation of the LDL receptor-related protein (LRP). *P. Greiser¹, S. Schulz^{1,3}, G. Birkenmeier², U. Schagdarsurengin¹, E. Archoukieh¹, U. Mueller-Werdan³, K. Werdan³, I. Hansmann¹, C. Glaeser¹.* 1) Inst. of Human Genetics, Univ. Halle, Halle, Germany; 2) Inst. of Biochemistry, Univ. Leipzig, Leipzig, Germany; 3) Dep. of Internal Med., Univ. Halle, Halle, Germany.

LRP, a multifunctional cell receptor is one of the most interesting candidate genes for degenerative cellular processes like Morbus Alzheimer, Parkinson and atherosclerosis. Methods: We investigated the human intraindividual (10 subjects, withdrawal once a week over a period of 4 weeks) and the circadian variation of LRP-mRNA-level (5 subjects, withdrawal at 8 am, 12 am and 4 pm) in native monocytes from healthy volunteers, respectively, by competitive RT-PCR and tested the influence of a novel promoter polymorphism (89 male subjects), and the effect of the adipocyte-derived hormone leptin (100 ng/ml for 24 hours) and the cytokine TGF- β 1 (5 ng/ml for 24 hours) on mRNA-expression (cell lines). Results: The measured intraindividual LRP-mRNA-level remained relatively constant regardless age or gender (SD: 0.12). The circadian rhythm of LRP-transcription showed gender specific differences: the increase during the day in female probands may be due to a sex hormone specific regulation. The males did not show such a remarkable circadian variation. The promoter mutation P C-25G (creation of a new binding site for the universal transcription factor SP1) led to a significant increase of the individual message expression: 149 vs. 110 ag/cell ($p < 0.048$). In all cell lines leptin had an upregulating influence on LRP-mRNA expression: endothelial cells - 11.8 to 24.8 ag/cell, monocytes (Mono-Mac-6) - 283.2 to 449.5 ag/cell, fibroblasts from skin - 1825 to 2898 ag/cell, fibroblasts from chorion - 1348 to 1544 ag/cell and keratinocytes (HaCat) - 438.7 to 481.6 ag/cell. Different effects on mRNA-level were measured after administration of TGF- β 1: carcinoma cell lines (downregulation: AsPc-1- 131 to 107 ag/cell, upregulation: LNCAP - 51 to 137 ag/cell, PC-3 - 181 to 652 ag/cell, DU-145 - 381 to 472 ag/cell) and skin fibroblasts (upregulation: 2754 to 8423 ag/cell). Our results suggest a complex transcriptional regulation of this multifunctional cell receptor in man.

The tissue-specific LMX1B gene is associated with a CpG island and is transcribed from a TATA-less promoter.

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The LIM-homeodomain transcription factor, LMX1B, is required for the development of the limbs, eyes, kidneys and portions of the CNS. Patients with heterozygous loss-of function mutations in the LMX1B gene develop Nail Patella Syndrome (NPS), which is typically characterized by nail dysplasia, patellar hypoplasia and elbow dysplasia. The severity of the phenotype varies greatly and can include nephropathy and Open Angle Glaucoma. It is hypothesized that the variation in severity reflects the level of LMX1B transcript/protein at critical points in development; therefore, we are interested in identifying cis-acting elements that control the spatio-temporal expression of LMX1B. Two regions of the human LMX1B gene were cloned for further study: 1) the 5' end of the gene including 5'flanking sequence and 2) the 3'UTR. 3.5 kb of sequence containing the putative transcription start site of the human LMX1B gene was subcloned from BAC 265A2. The insert was sequenced and tested for promoter activity in fibroblast cells. The TATA-less promoter directed transcription of a luciferase reporter gene at a level 10-fold greater than the empty vector. Additional sequence was obtained by searching the NCBI-htgs database. 15.5 kb of 5' sequence was analyzed for the presence of CpG islands with the GRAIL v1.3 program. In a GC-rich region spanning approximately 4 kb, multiple CpG islands were identified. The longest island (1.4 kb) spans exon 1 of the LMX1B gene. ESTs mapped to this region suggest that the CpG island is interrupted by a gene transcribed from the opposite strand relative to LMX1B. The CpG islands and EST sequence were conserved in mouse sequence obtained from the NCBI-htgs database. The terminal exon of the LMX1B gene was cloned from BAC 265A2 and sequenced. From this sequence, primers were designed for 3'RACE. RACE products indicate a 3'UTR of 4.57 kb. The 3'UTR sequence was searched for the presence of known motifs using the UTRscan program. No known motifs were detected. Identification of both human and mouse sequence containing the LMX1B promoter and 3'UTR will allow for comparative and functional analysis of cis-regulatory elements.

Analysis of the human alpha-synuclein promoter. *O. Chiba-Falek*¹, *B.M. Orrison*¹, *J.W. Touchman*², *A. Dehejia*³, *M.H. Polymeropoulos*³, *R.L. Nussbaum*¹. 1) GDRB, NHGRI, NIH, Bethesda, MD; 2) NISC, NIH, Gaithersburg, MD; 3) Novartis Pharmaceuticals, Gaithersburg, MD.

Mutations in the alpha-synuclein gene have been implicated in familial Parkinson's Disease (PD). In order to study the structure and regulation of the human alpha-synuclein gene, we sequenced the entire mouse and human genes, including ~20 kb upstream of the gene. A comparison between the human and the mouse sequence revealed several regions of sequence similarity over 11 kb upstream of the gene. We cloned from human PAC 27M7 a 10.7 kb fragment upstream of the translational start site, including the first two untranslated exons and the first intron, and used restriction sites to construct 7 additional constructs extending varying distances upstream of the gene; all 8 constructs were inserted in a luciferase expression vector and transfected into 293T and the neuroblastoma cell line SH-SY5Y. The shortest fragment, 400bp upstream of the transcriptional start site, was found to be sufficient for transcription in both cell lines. The other constructs led to variable expression levels, with some showing maximum expression and others showing nearly complete extinction of expression. There were also differences in relative expression level for each construct between the cell lines. These results illustrate that the different regions of the promoter might contribute to tissue specific up/down regulation of alpha-synuclein. Next, we constructed an alpha-synuclein "minigene" expression vector in which expression of the alpha-synuclein cDNA was driven by the full length 10.7 kb upstream region. Upon transient transfection into 293T cells, the minigene was transcribed and translated with high efficiency into the alpha-synuclein protein. This minigene provides an opportunity to study the regulation of alpha-synuclein expression both in cultured cells and in whole animals.

Nucleosomes in the *HPRT* promoter region are translationally phased on the active X chromosome and rotationally phased on the inactive X chromosome. *C. Chen*^{1,2}, *T.P. Yang*^{1,2,3}. 1) Dept Biochemistry & Molecular Biology; 2) Center for Mammalian Genetics; 3) Department of Pediatric Genetics, University of Florida, College of Medicine, Gainesville, FL.

Differential chromatin structure is one of the hallmarks distinguishing active and inactive genes. For the X-linked human hypoxanthine phosphoribosyl transferase gene (*HPRT*) this difference between alleles on the active and inactive X chromosomes is evident in both the general DNase I sensitivity and hypersensitivity of the promoter. Here we show that the differential chromatin structure of the active and inactive *HPRT* promoters involves alterations in nucleosomal positioning. Translationally positioned nucleosomes occupy the same DNA sequence in all cells, whereas rotationally positioned nucleosomes contact the same positions on the DNA helix in all cells. Using micrococcal nuclease digestion, we find translationally positioned nucleosomes on the active promoter except over a 350 bp region that includes all of the known transcription factor binding sites as well as the transcription initiation sites. Over this region, nucleosomes are either altered or absent based on increased sensitivity to both micrococcal nuclease and DNase I. In contrast, no translational positioning is evident on the inactive promoter and both DNase I and micrococcal nuclease hypersensitivity are absent. We also examined rotational positioning of nucleosomes in the *HPRT* promoter by high resolution DNase I *in vivo* cleavage analysis. This analysis reveals a strong 10 bp-laddering pattern consistent with rotationally positioned nucleosomes on the inactive minimal promoter but not on the active minimal promoter. In summary, we show differential nucleosomal positioning between the active and inactive alleles of the *HPRT* promoter, with the active promoter exhibiting translational positioning and the inactive promoter exhibiting rotational positioning.

Role of Human Chromosome Associated Protein-H (hCAP-H) in Chromatin Condensation. *W. He¹, S. Colicos¹, I.I. Ouspenski², J.W. Belmont¹, O.A. Cabello².* 1) Dept Molecular & Human Genetics, Baylor College of Medicine, Houston, TX 77030; 2) Department of Molecular and Cellular Biology, Baylor College of Medicine.

In the normal progression of the cell cycle, compaction of DNA from nucleosomes to high order loop structures is necessary for congression of condensed chromosomes in metaphase and for segregation of chromosomes in anaphase. Chromosome condensation requires the activity of a multimeric protein complex called 13S Condensin. Condensin is comprised of CAP-C and CAP-E ATPases that directly bind DNA and catalyze bending and supercoiling, and three other components of unknown function, CAP-D2, CAP-G, and CAP-H. When mutated, the *Drosophila* homolog of CAP-H, barren, gives a cellular phenotype suggesting failure of chromatid decatenation. The purpose of this project is to investigate the functional role of human CAP-H in mitosis and its more general roles in chromatin remodeling. One ongoing strategy for the analysis of mammalian CAP-H function consists of its targeted inactivation in mouse embryonic stem cells. A second approach is the elucidation of the functional domains of hCAP-H. Deconvolution microscopy indicates that in mitosis EGFP-tagged hCAP-H localizes in subchromosomal domains identical to those detected by immunofluorescence using anti-hCAP-H antibodies. Moreover, EGFP-hCAP-H (as well as CAP-E and CAP-C) localizes to the interphase nucleolus. Interspecies comparisons of CAP-H homologs highlight four conserved peptide motifs. Eight EGFP-tagged mutant forms of hCAP-H were prepared that selectively delete these conserved domains. These mutants have been studied for subcellular localization and effect on cell cycle progression. The N terminal domain of hCAP-H contains signals for nuclear localization not detected by motif analysis. The C terminal domain is required for localization to the nucleolus. Overexpression of full length hCAP-H leads to mitotic arrest, failure of progression to G2/M, and increased apoptosis in HeLa cells. Overexpression of C terminal domain mutants has a similar effect. Continuing characterization of these mutants and their relevant protein interactions should allow identification of the critical interaction domains required for Condensin function.

Molecular cloning and expression analysis of PAQ1/2, a transcriptional cofactor gene deleted in DiGeorge and velocardiofacial syndromes. E. CONTI^{1,4}, G. MITTLER², F. AMATI¹, L. BERTI², M. BENGALA¹, A. BOTTA¹, B. DALLAPICCOLA^{3,4}, G. NOVELLI¹, M. MEISTERERNST². 1) Dept. of Biopathology, University of Rome Tor Vergata, 00133 Rome, Italy; 2) Immunology of the GSF, Dept. for Proteinbiochemistry, D-81377, Muenchen; 3) Dept. of Exp. Med. and Pathology, La Sapienza University of Rome, I-00141 Italy; 4) CSS Mendel, Rome, Italy.

PAQ1/2 (PC2 associated Q-rich protein 1/2) is a component of a large multiprotein complex named PC2, highly related to the coactivator complexes TRAP/SMCC and DRIP/ARC. PAQ1/2 was identified during PC2 characterization by using a monoclonal antibody (IF8) directed against a polyglutamine stretch. PAQ1/2 protein is encoded by a gene of 79 Kb organized in 17 exons expressing a cDNA of 2241 bp. PAQ1/2 gene maps within the deletion interval 4 of the DiGeorge/Velocardiofacial (DGS/VCFS) critical region on chromosome 22q11.2. PAQ1/2 protein is constituted by 746 aminoacids and displays proline-rich domains, its calculated MW is 82,3 kDa while the apparent MW on SDS-PAGE is 105-107 kDa. The gene is deleted in the majority of patients with hemizigosity of the region resulting in haploinsufficiency of the product. PAQ1/2 is ubiquitously expressed in all investigated human tissues (RNA Master blot, Clontech). RT-PCR analysis revealed the presence of two different mRNA isoforms both in fetal and adult tissues. TRANSFAC and TFD analysis revealed that the 5' portion of the gene containing multiple transcription factor binding sites including, AP-2, Sp1, TFIID, GATA-1, GATA-2 and the absence of a TATA box. PAQ1/2 is a bridging factor for a variety of human regulatory proteins and could have an architectural role in the PC2-mediator complex; therefore, it is an excellent candidate for concentration-dependent perturbations of gene expression during development. Work supported by Telethon grants (E.723 and 364/bi).

Analysis of the transcriptional regulation of the human *HFE* gene. C. Mura, G. Le Gac, C. Ferec. Lab Biogenetique, INSERM, CHU, UBO, Brest, France.

Hereditary hemochromatosis (HHC) is the most common iron metabolism disorder in man. *HFE* gene, clearly involved in HHC, encodes a protein which interacts with the transferrin receptor (TfR) and should play a role in the regulation of iron uptake. *HFE* is widely expressed but the mechanism of the transcriptional regulation of the human *HFE* gene still remains to be clarified. The goal of the present study was to identify the *cis*-acting elements and the *trans*-acting factors involved in transcriptional regulation of the *HFE* gene expression. Transient transfection experiments were performed using a series of luciferase reporter pGL3 plasmid constructs containing various *HFE* 5'-flanking sequence. The *HFE* promoter-driven luciferase reporter gene activity was determined in three different human cell lines: hepatoma HepG2 cells, colon adenocarcinoma HT29-19A cells and epithelial HeLa cells. The *HFE*-promoter constructs displayed the greatest activity in HepG2 cells, followed by a significant activity in HT29-19A cells. The analysis of *HFE*-driven luciferase reporter gene activity indicated that the region extending from -253 to -6 relative to the first coding nucleotide contains a basal promoter region, and the -555/-6 segment showed the maximum activity. Cotransfection studies with *HFE*-promoter deletion constructs and vectors expressing transcription factors showed that the ubiquitous Sp1 and the liver-enriched protein GATA-1 can transactivate *HFE*-directed gene transcription with a maximum of 7.1-fold and 8.7-fold increase, respectively. This stimulating effect can be ascribed to their functional interaction with the presence of consensus binding sites in the *HFE* gene promoter; thus its expression appears to be regulated in a tissue-specific manner.

Direct transactivation of type X collagen by CBFA1 contributes to abnormal endochondral ossification in cleidocranial dysplasia. Q. Zheng, G. Zhou, Y. Chen, B. Lee. Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Mutations in the transcription factor *CBFA1* (core binding factor), a nonredundant determinant of osteoblast cell fate, cause the dominantly inherited cleidocranial dysplasia (CCD). CCD is characterized by hypoplastic clavicles, delayed closure of fontanelles, dental anomalies, and delayed skeletal development. It also involves long bones, especially in the hands and feet. Interestingly, *Cbfa1*^{-/-} mice have been shown to exhibit a defect in chondrocyte maturation. Based on these observations, we hypothesized that *Cbfa1* participates in chondrogenesis by activating type X collagen gene expression. The type X collagen gene (*Col10a1*) is specifically expressed in hypertrophic chondrocytes and mutations in *Col10a1* cause Schmid-type metaphyseal dysplasia. It was previously shown that 1.6 kb of the chicken *Col10a1* promoter in combination with the first intron contained elements sufficient to confer tissue specificity in transgenic mice. However, the corresponding sequence of murine type X collagen gene promoter failed to confer tissue-specific expression. We characterized a larger 4 kb promoter sequence in *Col10a1* both in cells and in transgenic mice. This 4kb promoter drove expression of a *LacZ* reporter weakly, but specifically to the hypertrophic chondrocytes in E15.5 and E18.5 transgenic mouse embryos. Sequence analysis of this promoter showed three potential CBFA1 binding sites at -3.5 kb (A), -2.4 kb (B) and -1.5 kb (C) prior to the transcription start site. Gel shift assays confirmed that they all bind to full length CBFA1 and its DNA binding runt domain. Furthermore, in transient transfection in COS-7 cells, CBFA1 transactivates a reporter gene which contained eight copies of the “A” enhancer element and a 50bp *Col10a1* minimal promoter upstream of the luciferase gene. Taken together, our *in vitro* and *in vivo* studies of mouse type X collagen suggest that its 4 kb promoter can confer tissue specificity and CBFA1 directly transactivates type X collagen gene. Hence, *CBFA1* mutations result in decreased *Col10a1* expression explaining the long bone dysplasia in CCD.

The Dp71 promoter activity is down-regulated during muscle cell differentiation. *M. Bermudez¹, C. Montanez¹, P. Gomez¹, D. Yaffe², B. Cisneros¹*. 1) Department of Genetics and Molecular Biology, CINVESTAV-IPN, Mexico city 07360, Mexico; 2) Department of Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel.

The Dp71 protein is a product of the Duchenne muscular dystrophy gene, which is present in many cell types and tissues. Detectable levels of Dp71 are found in non-differentiated muscle cells, but not in mature muscle cells. The Dp71 promoter is considered a "housekeeping" promoter because it lacks TATA and CAAT boxes, but contains several Sp1 binding sites and a putative initiator element that overlaps with the transcription start site. To study the regulation of the Dp71 promoter along muscular differentiation, a series of plasmids, carrying the wild type or different deleted promoter sequences driving the chloramphenicol acetyl transferase gene (CAT) were transiently transfected into C2C12 cells. Cells were differentiated by changing then to differentiation medium. Interestingly, the activity of the Dp71 promoter decreases 70% during muscle cell differentiation. The promoter region responsible for such regulation was mapped to 244 base pair fragment, which maintains the three Sp1 binding sites. Gel shift experiments prove that indeed Sp1 transcription factor binds to the Dp71 promoter. Since Sp1 transcription factor is developmentally down-regulated during muscle differentiation, it is possible that its diminish causes the drop of the Dp71 promoter activity. To test this hypothesis, transfection assays with a plasmid overexpressing Sp1 factor are currently in progress.

Up regulation of the RET gene expression by histone deacetylase inhibitor sodium butyrate: hints to the gene physiologic regulation and applications for mutations screening. *G. Patrone¹, F. Puppo¹, P. Griseri¹, G. Romeo², I. Ceccherini¹, R. Ravazzolo¹.* 1) Molecular Genetics Lab., G.Gaslini,Genova,Italy; 2) IARC, Lyon, France.

Histone acetylation is emerging as a major mechanism thought to modulate gene expression by altering the accessibility of transcription factors to DNA. Drugs inhibiting histone deacetylases have been shown to relieve repression of specific genes. In a previous study on the regulation of the RET gene expression, we showed that this gene transcription is cell-line specific, characterised the functional domains of its minimal promoter, and collected evidence that Sp1 is the main activator of RET promoter. This sequence did not exhibit cell-line specific activity. The apparent paradox of regulated levels of mRNA in the context of a housekeeping type promoter might be explained by the peculiar position of RET on chromosome 10, close to the third pericentromeric satellite. Under the assumption that chromatin conformation of this region might play a role in regulating RET transcription, we have started addressing the question of chromatin structure and histone modification within the RET locus. In this report we show that histone deacetylase inhibitor sodium butyrate can upregulate RET mRNA levels in both expressing and non-expressing cells. Reporter constructs containing the RET promoter display a 20 - 40 fold activation upon sodium butyrate treatment. We envisage promoter accessibility can be modulated by histone acetylation, and ubiquitous Sp1 might be subjected to tissue specific activity modulation. RET germline mutations account for 10-40% of patients affected with Hirschsprung disease (HSCR), a complex genetic disorder affecting intestinal innervation. Analysis of RET mRNA from HSCR patients has been hampered by the lack of this gene expression in blood cells. Sodium butyrate treatment has enabled us to analyse cDNA derived from blood cells: we report examples of detection of anomalous transcripts, defective gene expression in HSCR patients, as well as nucleotide substitutions in the coding region. Thus we have improved our potential to investigate the molecular basis of the disease.

Identification of a silencer element of the human acid maltase gene in normal human fibroblast cells and Glycogenesis type II fibroblast cells. *B. Yan, P. Plotz.* ARB, NIAMS/NIH, Bethesda, MD.

Acid maltase (acid alpha-glucosidase, GAA) is a lysosomal enzyme, deficiency of which results in a recessively inherited disorder-Glycogenesis type II. In our previous work, a 25bp silencer element and its binding transcriptional factors (Hes-1 and YY1) were identified in Hep G2 cells. We have now performed transient transfection experiments in normal human fibroblast cells (CRL1513) and fibroblast cells from an adult patient carrying a point mutation of the GAA gene at position -13 (t-g) relative to the intron1/exon2 boundary in intron 1. A series of intron 1 fragments were cloned in both orientations upstream of a reporter gene, CAT, under the control of a thymidine kinase (TK) gene promoter. Deletion analysis identified a silencer in a 25bp element which is located between 1711bp and 1735bp relative to the exon 1/intron 1 boundary. The significant repressive effect on TK promoter activity was 35-50% in both orientations in both normal and mutant fibroblast cells. The element is upstream of the previous silencer found in Hep G2 cells, and it is inactive in Hep G2 cells. Electrophoresis mobility shift assay (EMSA) showed that three proteins specifically bound to the element. Site-directed mutagenesis and functional analysis indicated that a 5bp motif (GGTAC) was essential to the silencer function. Mutation of the motif abolished the repressive effect entirely in both normal and mutant fibroblast cells. The precise identification of the responsible proteins binding to the element is under investigation. The data may be helpful for designing a therapy to overcome the most common genetic defect in adult patients with the disease: reduced production of structurally normal enzyme caused by the mutation (-13 t-g) that reduces the efficiency of normal splicing.

Analysis of the role of the branchpoint A of CFTR intron 8 upon alternative splicing. *T. Hefferon, E. Niemitz, M. Egan, G. Cutting.* Human Genetics, Johns Hopkins University School of Medicine, Baltimore, MD.

Mis-splicing of CFTR transcripts, which results in reduced levels of full-length mRNAs, has been associated with cystic fibrosis, atypical CF, and congenital bilateral absence of the vas deferens (CBAVD). The 5T allele at the splice acceptor of intron 8 of the CFTR gene is found in 10% of the general population and has been shown to contribute to the aberrant splicing of exon 9. The 5T allele displays variable penetrance which has been hypothesized to be due to the distance of the branchpoint A from the 5T mutation; the intervening sequence consists solely of a TG dinucleotide repeat. To investigate the role of the branchpoint A in alternative splicing at this locus, we created a minigene construct containing parts of exons 8 and 10 and all of exon 9, including flanking intronic sequences, fused in-frame to an ornithine-amino-transferase (OAT) cDNA, and transfected it into mammalian cells. Splicing efficiency was evaluated using RT-PCR and capillary electrophoresis; we controlled for overamplification of shorter products by use of a correction factor determined by the amplification of a control plasmid containing one copy each of exon 9- and exon 9+ targets. HEK293 cells transfected with the minigene construct carrying the 9T allele spliced approximately 99% of minigene transcripts fully (n=3), whereas one carrying a shorter polypyrimidine tract, the 5T allele, spliced with only 70% efficiency (n=3). These results replicate observations of transcripts derived from 9T and 5T alleles in vivo and support the use of this system as a model for studying splicing at this locus. Then, to evaluate the role of the branch A in alternative splicing, we mutated the putative branch A to a G (on a 9T background) in the minigene construct, and found that splicing of transcripts derived from this plasmid was unperturbed (97% efficiency, n=2). We suggest three possible explanations for these observations: 1) use of an alternative branchpoint A nearby when the primary branchpoint has been eliminated; 2) use of the substituted G as a novel branchpoint target, or 3) the putative branchpoint A is not involved in splicing of exon 9.

An RBMX-like gene selectively expressed in testis and located at 9p24. *P.A. Lingenfelter¹, H.E Hoekstra², D.L. Delbridge³, J.A. Marshall Graves³, C.M. Disteche¹.* 1) Dept Pathology, Univ Washington, Seattle, WA; 2) Dept of Zoology, University of Washington, Seattle, WA; 3) Dept of Biochemistry and Genetics, La Trobe Univ., Melbourne, Australia.

RBMX is an X-linked gene homologous to RBMY, a Y-borne gene implicated in male fertility. Both genes encode for proteins involved in RNA splicing. We previously reported that several RBMX-like sequences existed in the human genome. These copies are all processed retroposons. Some of the copies are expressed in different tissues. We report here that one RBMX copy is located at band 9p24 and is selectively expressed in testis and to a lesser extent in brain. This copy of RBMX is conserved in primates. Interestingly the region of chromosome 9p24 where this gene is located is deleted in patients with 46,XY sex reversal. Although DMRT1 has been proposed as a candidate for this type of sex reversal, the testis-specific RBMX-like sequence we describe may also play a role in normal sexual development, specially in view of its potential role in tissue-specific splicing. Alternative splicing has been shown to play a critical role in sex determination of model organisms such as *Drosophila*. Another role for the RBMX-like gene at 9p24 would be to provide RBMX-like expression in the testis when RBMX is inactivated on the X chromosome. This would be analogous to other X-linked genes which have processed copies on autosomes which are selectively expressed in testis.

Nramp2 ANALYSIS IN HEMOCHROMATOSIS PROBANDS. *G. LE GAC*¹, *C. MURA*², *A.Y. MERCIER*¹, *O. RAGUENES*³, *C. FEREC*¹. 1) EFS-Bretagne, Brest, France; 2) UBO, Brest, France; 3) CHU, Brest, France.

The mechanism that leads to iron overload in hereditary hemochromatosis (HHC) is not yet fully understood and other genes than HFE may be involved. Nramp2 is an intestinal iron transporter, upregulated by dietary iron deficiency, and which also co-localizes with transferrin in recycling endosomes. The purpose of the present study was to analyse the coding region of the Nramp2 gene in hemochromatosis probands (HC) without any HFE mutation on both chromosomes. RT-PCR was designed to encompass the entire coding region of the Nramp2 gene and amplification was achieved from peripheral blood leukocytes of fourteen HC probands and eighteen control subjects. Then, sequencing analysis of the RT-PCR products allowed us to detect one single nucleotide polymorphism (a TC change at nucleotide 1254 in the cDNA) presumably not associated with HHC, but no mutation. On the other hand, we identified seventeen splicing forms of the Nramp2 mRNA: five of them corresponded to exons skipping and the twelve others to utilisation of cryptic splicing sites. Systematic genomic DNA sequencing of the corresponding exon-intron borders and the sequences surrounding the cryptic splicing sites did not reveal any mutation liable to result in aberrant splicing. Thus, these seventeen splice variants are likely alternative splicings. Eight of them were especially detected in most HC probands and control subjects. They corresponded to activation of cryptic splicing sites, which match consensus splicing sequences, between exons 3 and 4. This indicates existence of an important splicing instability in this region. In conclusion, our study did not give evidence for the involvement of the Nramp2 gene in hereditary hemochromatosis. However, the biological significance of the splicing instability revealed between exons 3 and 4 is unclear and requires thorough investigations to be understood.

An IVS32+1G®A mutation in a type I collagen gene, *COL1A2*, results in use of a rare AT/AC splice system and may ameliorate the osteogenesis imperfecta phenotype caused by exon-skipping. C.D. Kuslich, P.H. Byers. Dept Pathology, Univ Washington, Seattle, WA.

The type I collagen gene *COL1A2*, like most human genes, uses the U2 spliceosome and 5' GT / 3' AG splice junctions in each intron. A mutation of any of these four nucleotides leads to aberrant splicing. We identified a substitution that creates a 5' AT donor site and leads to use of a cryptic 3' AC acceptor site in IVS32 of the *COL1A2* gene, in an individual with moderately severe osteogenesis imperfecta (OI type III/IV). Total cellular and nuclear RNA were isolated separately from cultured dermal fibroblasts cultures, DNase I treated, and cDNA was synthesized. The *COL1A2* transcript was amplified in four overlapping fragments by RT-PCR using total cellular RNA. In a fragment spanning exons 31-44, the expected 1038 bp fragment and a 930 bp product, which lacked exon 32, were seen. Genomic DNA was heterozygous for an IVS32+1G®A mutation. When nuclear derived cDNA was amplified with primers in exons 28 and 33 an additional heteroduplex was seen that contained both the normal sequence and one with the last 6nts of IVS32 inserted (TTTCAG). This insertion was created by use of the new 5' splice site, ATACGT, and a cryptic 3' splice site TAC, adjacent to the 6 bp insertion. AT-AC intron termini are used in a minor U12 splicesomal pathway, which has stringent intron sequence requirements not met by the *COL1A2* mutant allele. Two human genes, *SCN4A* and *SCN5A*, use U2-dependent AT-AC intron termini. The 5' splice site sequence for *SCN5A* (AG/ATACGTAG) differs by one nucleotide from the new *COL1A2* sequence (AG/ATACGTGT). These findings imply that a rare AT-AC spliceosome pathway removes all but 6nts of IVS32 from some transcripts diminishing the amount of the exon 32-skipped product, which may ameliorate the expected severe outcome of this mutation. Use of the AT-AC splice sites in this intron in addition to exon 32 skipping may indicate IVS32 is quickly removed promoting exon 32 skipping for many transcripts while for others IVS32 is spliced slower permitting the use of the of the AT-AC mechanism. Similar mutations in other genes may be more common than expected. (NIH AR21557, AR41223).

Alternative splicing of DNA Polymerase β mRNA in normal tissues and bladder cancer. *T.E. Thompson¹, P.K. Rogan², J.I. Risinger¹, J.A. Taylor¹.* 1) Lab Mol Carcinogenesis, NIEHS, Research Triangle Park, NC; 2) Sect Med Genet & Mol Medicine, Children's Mercy Hosp, Univ Missouri-Kansas City School of Medicine.

Large intragenic somatic deletions of DNA Polymerase β (POL β) have previously been inferred from analysis of tumor cDNAs. We noticed that the breakpoints of these presumed rearrangements were delineated by exon-exon junctions, which could instead be consistent with alternative splicing. Genomic DNA of bladder tumors and bladder cancer cell lines was screened for mutations. Deletions and somatic mutations were not seen, although 2 polymorphisms were detected. We then analyzed cDNA from the cell lines and a subset of tumors with matched normal tissue. A diverse array of alternative splice variants was found, even within the same samples. Alternative splicing is somewhat more common and variable in bladder cancer cell lines and tumor tissues than in normal bladder. In alternative splicing, the upstream acceptor that is skipped generally exhibits weaker splicing signals than the downstream acceptor site which is selected. Since the sequences of acceptor sites in POL β were unaltered, we explored the possibility that differences in splice site strength, ie. information content (R_i), could account for the alternative splicing variants. The R_i values of alternatively spliced and skipped acceptor sites were compared with those of the corresponding constitutively spliced acceptors. In most alternative splicing events (41 of 49 cDNAs; exons 2, 4, 4-6, 9, 2-13, 7-9 or 4-13 skipped), the R_i value of the acceptor site that was selected exceeded that of the upstream acceptor had splicing been constitutive. Differences between the strengths of these pairs of sites ranged from >1.3 to 36 fold, with the most common variant (lacking exon 2) exhibiting > 22.6 fold difference. By contrast, R_i values of downstream acceptor sites did not exceed those of the corresponding upstream sites in alternative splicing events where either exons 2-6, 4-5, 5-6, 7-8 or 11 were skipped. Interestingly, 6 of 8 cDNAs derived from these splice forms occurred in tumor cell lines. There appears to be loss of specificity in tumors for alternative splice forms in which splice site strength dictates splice site use.

Molecular dissection of the Schwann cell specific promotor of the PMP22 gene. *M. Fontes, D. Saberan-Djoneidi, V. Sanguedolce, N. Levy, E. Passage.* INSERM U491, Medical Genetics and developmentl, Fac de Medecinede la Timone, Marseille Cedex 5, France.

PMP22, one of the major components of myelin, is overexpressed in Charcot-Marie-Tooth type 1A (CMT1A) patients. In an attempt to determine the mechanisms of regulation of the expression of this gene (and potentially to lower its expression in CMT1A patients), we subcloned genomic fragments from the promotor, up to 6 kb, in an expression vector containing the b-galactosidase gene as reporter, and used it in a transfection assay. We determined that only 300 bp upstream of the transcriptional start are sufficient to promote the Schwann cell specific expression of the reporter gene. This minimal promotor seems to be under the control of a silencer element sensitive to cAMP, located between -0.3 kb and -3.5 kb from the start of transcription. We sequenced and analyzed 2 kb of the promotor using computer programs, which have predicted the existence of potential binding sites for transcription factors binding sites, such as CREB (which is probably involved in the response of PMP22 expression to cAMP stimulation) and steroid receptors. Using constructions containing or not the CREB sites, we were able to demonstrate that these sites are involved in the silencing process of the PMP22 promoting activity. Finally, we found a region containing blocks of polymorphic CA repeat, located close to the CREB binding site, which can potentially influence the transcriptional activity of this gene.

Function of *Distal-less* subfamily isoforms, BP1 and DLX7, in the repression of human b-globin gene. *S. Fu*¹, *J.W. Strovel*², *S.B. Haga*², *J. Stamberg*², *P.E. Berg*¹. 1) Dept. of Biochemistry & Molecular Biology, George Washington Univ Med Ctr, Washington, DC; 2) Div. of Human Genetics, University of Maryland, Baltimore, MD.

Beta Protein 1 (BP1), a new homeobox (HOX) cDNA encoding a transcription factor, appears to be involved in repression of the human b-globin gene. Sequence comparison revealed that BP1 is a HOX gene, which shares the same HOX domain with human *Distal-less* gene DLX7 and DLX4. DLX7 and DLX4 have been mapped to chromosome 17q21-22. In order to explore the possible relationship of BP1, DLX7 and DLX4, we mapped BP1 using fluorescence in situ hybridization (FISH) and PCR. One positive clone was obtained by screening a human bacterial artificial chromosome (BAC) genomic library using a 360bp BP1 unique region cDNA probe. The total BAC genomic DNA was direct-labeled and FISH analysis was performed. The BP1 gene was localized to chromosome 17q by dual hybridization with this probe and a labeled whole chromosome 17 library. To precisely define the map location of BP1, we successfully amplified DLX7 from this BAC clone using PCR. These results indicate that BP1 and DLX7, DLX4 are isoforms since they map to the same locus and exhibit extensive regions of sequence homology. Gel shift analysis of BP1 and DLX7 proteins after *in vitro* transcription and translation shows that they both bind to Silencers I and II located upstream of the b-globin gene. To compare the functions of BP1 and DLX7, we cloned both BP1 and DLX7 open reading frames into eukaryotic expression vectors. Subsequent transfection of BP1 and DLX7 respectively into the human erythroleukemia cell line K562 was carried out to determine the role of DLX7 in b-globin gene expression. Interestingly, contrary to BP1, overexpression of DLX7 does not repress b-globin gene expression. Nevertheless, both BP1 and DLX7 proteins bind to Silencer I and II *in vitro*, but only BP1 shows functional activity *in vivo*. Therefore, further characterization of these isoforms is of significance to the understanding of the control and balance of b-globin gene during early development.

Spastin, a new AAA protein, binds to α and β tubulins. *A.C. Azim, A. Hentati, M.F.U. Haque, M. Hirano, K. Ouachi, T. Siddique.* Neurology, Northwestern Medical School, Chicago, IL.

Autosomal dominant hereditary spastic paraplegia (AD-HSP) is a heterogeneous neurodegenerative disorder. This involves progressive and bilateral spasticity of lower limbs. Four genes responsible for this condition have been localized on chromosomes 14q, 2p, 15q and 8q. The SPG4 locus on 2p22 accounts for 40-50 % of AD-HSP families and is caused by mutations in spastin gene. We have identified five novel mutations in spastin gene. Spastin is a 616 amino acid long protein and has homology to AAA family proteins. Spastin also has homology to a known microtubule severing protein, Katanin. In order to address the function of spastin protein we made three spastin-GST-fusion proteins of different lengths and developed a GST-spastin pull down assay using total human platelet protein as the source for α and β tubulin. Bound tubulin was visualized using anti tubulin monoclonal antibodies. The first construct corresponding to amino acid (1-264) binds to tubulin more specifically. The c terminus GST-spastin clone also showed binding to α and β tubulin, suggesting that there are two microtubule binding site in spastin, each located in the two halves of the protein. Glutathione S-transferase was used as a control and showed no binding at all to α and β tubulin. Experiments are underway to explore the specificity and affinity of this interaction. We are also developing reagents to test direct interaction of spastin and tubulin. Spastin also has a consensus nucleotide binding P-loop. Experiments are underway to explore expected nucleotide binding, ATP-ase activity, microtubule severing, and the effect of spastin human mutations on its interaction with tubulin.

JAG1 missense mutations in Alagille syndrome. *J.J. Dowhanick-Morrisette, R.P. Colliton, K.A. Lantz, N.B. Spinner.*
Dept Human Genetics, Children's Hosp Philadelphia, Philadelphia, PA.

Alagille syndrome (AGS) is a developmental disorder caused by mutations in Jagged1 (JAG1), a cell surface ligand in the Notch signaling pathway. JAG1 interacts with the Notch transmembrane receptor to release an intracellular form of Notch that stimulates transcription. JAG1 mutations are found in 60-70% of AGS patients. The majority of these mutations are protein truncating and 14% are missense mutations, however, no genotype-phenotype correlation has been observed. Over 30 missense mutations have been identified. Twenty are at the N-terminus of the protein, 9 are in the EGF-repeats and 1 is in the cysteine-rich domain. The precise mechanism by which Jagged1 and the Notch receptors interact is unknown. We are studying missense mutations in AGS patients to determine whether they are disease causing and how these single amino changes effect protein function. Four missense mutations (L37S, P163L, R184H, and P871R) were introduced into JAG1 by site-directed mutagenesis, cloned into a retroviral expression vector and expressed in NIH 3T3 cells. In order to test mutant JAG1 function, we used an assay to measure JAG1 induced activation of transcription via release of a Notch sensitive repression complex. In the presence of JAG1 signaling, transcription from the sensitive promoter can be measured. Both JAG1-L37S and JAG1-R184H were unable to activate the Notch signaling system, while P163L and P871R were comparable to wild-type JAG1. Intracellular localization studies demonstrated that L37S and R184H were not present on the cell surface. Furthermore, R184H is abnormally glycosylated which may result in improper trafficking through the ER and Golgi, preventing expression on the cell surface. JAG1-P163L and P871R and wild-type JAG1 were correctly localized. This is consistent with these latter two mutations being normal variants of JAG1. Missense mutations clustered at the N-terminus of Jagged1 may lead to defects in proper targeting of the protein to the cell surface. Studies involving missense mutations located in the EGF-repeats and cysteine-rich domain are in progress and may provide insight into other mechanisms for inactivation of Jagged-Notch signaling.

Transfer of the complete 2.4 Mb human DMD-gene to mice; expression analysis and crossing with *mdx* mice. *J.T. Den Dunnen, R.G. Maatman, E.J. De Meijer, B.M. Van Rijn, A.J. Verkerk, G.J.B. Van Ommen.* Human and Clinical Genetics, Leiden University Medical Center, Leiden, Nederland.

Starting from overlapping YACs, we have reconstructed the full 2.4Mb human DMD gene in yeast. For transfer of the gene to murine LA9 and ES cells we tried two approaches, direct transfer and as a mammalian artificial chromosome (MAC). Assuming that for a functional MAC the DMD gene itself would provide length and replication origins, we have re-modelled the original YAC by adding 150 kb of Y-centromeric DNA, human telomeric sequences and mammalian selectable markers. After yeast spheroplast/mammalian cell fusion clones were obtained of which four LA9 and two ES clones contained all DMD exons. FISH analysis showed that MAC-derived clones often contained extra-chromosomal structures, however, these were present in variable copy numbers and upon non-selective culturing were either gradually lost or integrated. To assess integrity and proper transcription, the full-size DMD clones were analysed by RT-PCR and subjected to MyoD-induced myo-differentiation. Subsequently, transgenic mice were generated and subjected to detailed phenotypic and expression analysis. RT-PCR, Western blot analysis and immuno-histochemical studies showed that the human DMD gene had the proper expression of muscle-specific and ubiquitous dystrophin isoforms. To study whether the human DMD gene was able to functionally complement a murine DMD mutation, the mice were crossed with *mdx* mice. Currently, we are analysing these mice and their offspring using several techniques, including expression profiling using cDNA chip and micro-array technology. The results of these studies will be reported. In combination with a DMD-gene deficient mouse which we are generating, we aim to breed a "dystrophin-humanized" mouse which should facilitate human-specific functional and therapeutic studies.

Structure-Function Analysis of Alternate Substitution Mutations at Codon 892 of the Human Androgen Receptor Associated with Complete Androgen Insensitivity. *Y.A. Elhaji*^{1,4}, *R. Lumbroso*⁴, *D.R. Foerster*⁵, *C.R. Greenberg*⁵, *K. Wrogemann*⁵, *L. Pinsky*^{1,2,3,4}, *L.K. Beitel*⁴, *M.A. Trifiro*^{1,2,3,4}. 1) Human Genetics; 2) Medicine; 3) Pediatrics, McGill University; 4) Lady Davis Institute, JGH, Montreal, PQ, Canada; 5) Biochemistry & Medical Genetics, University of Manitoba, Winnipeg, Canada.

The human androgen receptor (AR), a member of the nuclear receptor superfamily, acts as a ligand-dependent transcription factor. AR mutations may result in varying degrees (ranging from mild to complete) of androgen insensitivity syndrome (AIS). The AR contains a polymorphic N-terminal region that modulates transcriptional responses, a DNA-binding domain, and a C-terminal ligand-binding domain (LBD). Here we describe two novel mutations in the LBD of the AR, in unrelated patients with complete AIS, that result in substitution of two different amino acids (A and L) for a proline at an identical location: position 892. Kinetic analysis of the mutant receptors in cultured GSF and in transfected COS-1 cells revealed reduced total binding (B_{\max}) and increased dissociation (k_{diss}) and equilibrium affinity constants (K_d) for mibolerone (MB). However, with methyltrienolone (MT; R1881), K_d values were elevated for P892A and normal for P892L. The proline at position 892 is immediately adjacent to the N-terminus of helix 12 (H12) of the LBD. H12 contains the transcriptional activation function-2 (AF-2) core and is important for the recruitment of and interaction with transcription intermediate factors. H12 is also involved in AR N/C terminal interactions. Upon binding androgen, H12 is thought to undergo a distinct repositioning, forming a lid over the ligand-binding pocket and exposing a coactivator recruitment surface. We postulate that substitutions at position 892 alter the precise repositioning of H12, allowing for faster ligand dissociation. This hypothesis may explain the kinetics of both mutant ARs. Investigation of different substitutions at a single position of the AR provides a fine tool for detailed AR structure-function analysis and understanding of the mechanisms by which normal and mutant ARs regulate androgen-responsive genes.

Binding of L1 ORF1 protein to RNA using in vitro studies. *V. Kolosha, S. Martin.* Department of Cellular & Structural Biology, University of Colorado School Medicine, Denver, CO.

LINEs are long interspersed nuclear elements that are abundantly present in the mammalian genome. They belong to the non-long terminal repeat (non-LTR) class of retrotransposons, which contain a 3' poly(A) tail. Full-length mouse and human LINE-1 elements contain two open reading frames that encode proteins essential for retrotransposition. ORF1 protein is implicated in both protein-protein and protein-single stranded nucleic acid interaction. These functions of the ORF1 protein are likely to be important for ribonucleoprotein complex (RNP) assembly. Using both baculo- and bacterially- expressed ORF1 protein we reproducibly co-immunoprecipitated the largest T1 nuclease fragment (38nt) from mouse L1 RNA which is positioned just 3' of the initiation codon for the ORF2 region. However, further studies revealed that short transcripts containing this region, either in sense or in complement orientation, are efficiently co-immunoprecipitated. There is an apparent length-dependence for the co-immunoprecipitation of RNA fragments. Studies using nitrocellulose filter binding assay also did not detect any sequence specificity of the ORF1 protein binding to RNA. ORF1 protein binds long transcripts containing the 38 nucleotide region and its antisense with an apparent dissociation constant of approximately 75nM in 100mM NaCl. Based on UV-crosslinking studies the C-terminal 1/3 of ORF1 protein is the region involved in RNA binding. The affinity for the C-terminal portion of the protein under similar conditions is more than 10-fold less than for the full-length protein. This is likely to be closer to the real association constant for the RNA-protein interaction, since the C-terminal protein does not have the protein-protein interaction domain, that contributes to the cooperativity of binding. The binding of the protein to RNA is likely explained by electrostatic interaction of its highly positively charged C-terminal portion with the negatively charged phosphodiester backbone of RNA. Consistent with this, there is a strong salt dependence of RNA binding by the ORF1 protein.

Comparative sequencing of RP1 to identify evolutionarily conserved regions of the protein. The RP1

Consortium. *K.A Malone*¹, *J. Zuo*², *D.B. Farber*³, *J.R. Heckenlively*³, *E.A. Pierce*⁴, *C.F. Inglehearn*⁵, *S.P. Daiger*¹, *L.S. Sullivan*¹. 1) Human Genetics, University of Texas-Houston, Houston, TX; 2) St.Jude Children's Research Hospital, Memphis TN; 3) Jules Stein Eye Inst. UCLA; 4) Scheie Eye Inst., Univ of Penn; 5) Molecular Medicine Unit., Leeds Univ. UK.

Retinitis pigmentosa (RP) is a group of inherited retinopathies that is a leading cause of inherited blindness worldwide. Mutations in the RP1 gene may account for approximately 10% of all autosomal dominant retinitis pigmentosa (adRP), yet to date, there is limited structural or functional knowledge about the RP1 protein. Database searches have revealed significant sequence similarities with members of the doublecortin family of proteins, as well as a retina-specific EST, EYE2931, but the similarity extends only throughout the first 300 amino acids of RP1. The remaining 70% of the protein has no significant similarity to any known protein. In order to identify evolutionarily conserved regions of the protein, which are likely to be functionally important, we have sequenced the coding region of the RP1 protein in several different species of mammal. We used comparative sequencing as a way to predict which regions of the gene may be important for normal function or stable protein structure, and for examining the potential effects of missense mutations, polymorphisms, and benign variants. Several of the possible functional domains identified in human RP1 such as transmembrane domains, cell attachment signals, a leucine zipper, and an NDP kinase active site are not conserved in other mammals. Two of the three nuclear localization signals are conserved in baboon, squirrel monkey and mouse. The leucine zipper is not conserved in mouse or squirrel monkey, and the NDP kinase active site is not conserved in squirrel monkey. The exceptional amount of sequence divergence in RP1 is unusual for a seemingly critical retinal protein, and may explain why only large disruptions in the protein sequence appear to cause retinal disease.

The Sonic Hedgehog signaling pathway: analysis of the *Hedgehog Interacting Protein* and *HNF-3b* genes as potential causes of holoprosencephaly. *L. Huo*¹, *E. Roessler*¹, *P.T. Chuang*², *A.P. McMahon*², *M. Muenke*¹. 1) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Department of Molecular and Cellular Biology, The Biolabs, Harvard University.

The Hedgehog-Interacting Protein (HIP) is a novel component of the Sonic Hedgehog (SHH) signaling pathway. Recently, defects in the cell signaling pathway involving SHH have been shown to cause HPE, which is the most common birth defect of the brain and face in humans. In animal models, knockout mice with homozygous null mutations for *Shh* displayed abnormalities consistent with HPE. Hip has been shown to attenuate Hedgehog signaling by cell-surface binding of the hedgehog protein and to act in a negative regulatory feedback loop. Although Hip has been considered an important factor in the Shh signaling pathway, its potential role in HPE had not been examined in humans. Here we report for the first time the complete gene structure of the human *HIP* gene and present its mutational analysis in HPE patients. No mutations were found in the entire coding region or 1 kb upstream 5' of transcription start site (determined by 5'-RACE and primer extension analysis) suggesting a limited role for this gene in HPE pathogenesis. We are currently working on HNF-3b which is a transcription factor which acts as regulator of floor plate development whose induction depends on Sonic Hedgehog signaling. Preliminary mutational analysis of the *HNF-3b* gene in HPE patients is presented.

The role of PDZ-binding motif in polarized distribution of CFTR and other apical or basolateral membrane proteins. *M.I. Milewski¹, J.K. Forrest¹, J.E. Mickle¹, B. Stanton², G.R. Cutting¹.* 1) Inst Genetic Medicine, JHU Sch Med, Baltimore, MD; 2) Dept Physiology, Dartmouth Med Sch, Hanover, NH.

C-terminal PDZ-binding motifs are required for polarized apical/basolateral localization of many membrane proteins. However, apparent sequence similarities observed between PDZ-binding motifs of some apical and basolateral membrane proteins suggest that the specific subcellular localization of these proteins cannot depend solely on the PDZ domain-based interactions. We have previously shown that the PDZ-binding motif of cystic fibrosis transmembrane conductance regulator (CFTR), a protein mutated in cystic fibrosis (CF), is a part of a multi-component C-terminal localization signal responsible for apical protein distribution in epithelial cells. To further explore the function of this PDZ-binding motif we utilized the green fluorescent protein (GFP) fused to the CFTR C-terminal region containing all signals required for apical membrane localization in polarized MDCK cells. Substitution of the PDZ-binding motif of CFTR (D-T-R-L>) with E-T-H-F>, a motif from the apically located protein GAT3 did not alter apical distribution of the fusion protein. Interestingly, substitutions with PDZ-binding motifs from basolateral proteins BGT1 (E-T-H-L>) or LET23 (E-T-C-L>) did not affect the apical localization of the fusion protein. The fusion proteins containing PDZ-binding motifs of two other basolateral proteins GLUT1 (D-S-Q-V>) or SYN2 (A-F-Y-A>) showed diffuse cytoplasmic distribution and no specific basolateral or apical localization. These results strongly suggest that PDZ-binding motifs are auxiliary components of apical/basolateral localization signals that might be required for stabilization of protein at given location but do not determine its specific subcellular distribution.

Characterization of the Promoter of Human LST1: A Small Gene with a Complex Pattern of Alternative Transcripts. *X.F. Yu, S.M. Weissman.* Dept Genetics, Yale Sch Medicine, New Haven, CT 06519.

The gene for the human leukocyte-specific transcript 1(LST1) encodes a small protein that modulates immune responses and cellular morphogenesis. LST1 transcripts are expressed at high levels in dendritic cells. Because of its complex splicing pattern, use of alternative 5'-UT exons, and biologically interesting pattern of expression, we studied the human LST1 gene promoter and regulatory elements. We identified an additional upstream 5'-UT exon and found that each of the five alternative first exons was expressed from the endogenous in U937 monocytic cells. Transient transfection studies demonstrated that the combination of regions from -1363 to -621 with -112 to -54, relative to the translation start codon, produced the highest level of transcripts from among the various constructs tested, but the pattern of transcripts produced was only a subset of those produced from the endogenous gene. DNase I footprinting analysis and electrophoretic mobility shift assays (EMSA) showed that oligonucleotide probes corresponding to three regions, -1171 to -1142 (BI), -1136 to -1111 (BII), and -783 to -751 (BIV), bound proteins in U937 nuclear extracts. Competition and super-shift EMSA did not identify any known transcription factors responsible for BII probe binding. These studies suggest that a novel DNA binding site and interaction of multiple regulatory elements may be involved in mediating the expression of the various forms of LST1 mRNA.

A new paramyotonia congenita mutation in a novel Na⁺ channel region involved in inactivation. *F. Wu^{1,5}, M. Takahashi², E. Pegoraro³, C. Angelini³, P. Colleselli⁴, S. Cannon², E. Hoffman⁵.* 1) University of Pittsburgh, Department of Human Genetics, Pittsburgh, PA; 2) Harvard University, Department of Neurology, Boston, MA; 3) University of Padova, Department of Neurological and Psychiatric Sciences, Padova, Italy; 4) Divisone di Pediatria, ULSS n.1 Belluno Agordo Cadore, O.C. di Belluno, Belluno, Italy; 5) Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC.

Paramyotonia Congenita (PC) is an autosomal dominant non-progressive disorder showing cold-induced myotonia and transient muscle weakness, and is caused by gain-of-function missense mutations in the skeletal muscle sodium channel gene (SCN4A). Most PC mutations cluster in a voltage sensing region of the channel, and result in fast inactivation defects. Single-strand conformation polymorphism (SSCP) was used to screen all 24 exons of SCN4A and conformers were sequenced to confirm the presence of mutations. A new SCN4A mutation was introduced into a mammalian expression construct using site-directed mutagenesis method. Whole-cell recording from transiently transfected human embryonic kidney (HEK) cells was performed to determine electrophysiological characteristics of mutant channels. A new three generation Italian family segregating PC is presented, in which we identify a novel SCN4A mutation (L266V). This change removes only a single methylene group from the 1,836 amino acid protein, and is present in a region of the protein previously not known to be critical for channel function (domain I transmembrane segment 5). Electrophysiological studies of the L266V mutation showed defects in fast inactivation, consistent with other disease-causing SCN4A mutations studied to date. This change showed no significant abnormalities in slow inactivation. This novel mutation of the sodium channel indicates that a single carbon change in a transmembrane alpha-helix of domain I can lead to dramatic changes in channel inactivation and cause paramyotonia congenita.

Alternative splicing regulates the nuclear or cytoplasmic localization of dystrophin Dp71. *E. Gonzalez-Rodriguez¹, C. Montanez-Ojeda¹, P. N. Ray², P. L. Howard², F. Garcia-Sierra³.* 1) Genetic and Molecular Biology, CINVESTAV, Mexico, Mexico DF; 2) Department of Molecular and Medical Genetics University of Toronto, Canada; 3) Department of Cell and Molecular Biology. Northwestern University Medical School.

To get insight into the functional role of Dp71 alternative splicing, we determined the pattern of subcellular distribution of Dp71 isoforms alternatively spliced for exon 71 and/or 78. The cDNA sequence of each Dp71 variant was fused to the C-terminus of the green fluorescent protein (GFP). GFP-Dp71 gene fusion plasmids were transfected transiently in three different cell lines (HeLa, C2C12, and N1E-115) and the subcellular distribution of the fused proteins was determined by confocal microscope analysis. The Dp71 variant lacking amino acids encoded by exon 71 and conserving those encoded by exon 78 completely localizes to the nucleus whereas the variant lacking the amino acids encoded by both exons 71 and 78 was found exclusively in the cytoplasm. The remaining two isoforms generated from constructs containing exon 71 and exon 78 or preserving exon 71 but not exon 78 show a predominant nuclear localization. These results indicate that alternative splicing of Dp71 modulates its subcellular localization and that the presence of amino acids encoded by either exon 71 or exon 78 enables Dp71 to migrate into the nucleus. The nuclear localization of Dp71 provides a new clue towards the establishment of its cellular function.

Cell Cycle dependent phosphorylation of the murine homologue of the Treacher Collins Syndrome gene product, treacle. *S. Rujirabanjerd*¹, *S.T. Winokur*², *R. Shiang*¹. 1) Dept Human Genetics, MCV, Virginia Commonwealth Univ., Richmond, VA; 2) University of California, Irvine, CA.

Treacher Collins Syndrome (TCOF1) is an autosomal dominant disorder of craniofacial development. Although the gene has been cloned, the functional and biochemical properties of the protein, treacle, are still unknown. Treacle is thought to be a member of a class of nucleolar phosphoproteins from its predicted protein sequence. The murine homologue of the TCOF1 gene has also been identified. The murine treacle is 1320 amino acids in length and contains several potential sites for CKII (casein kinaseII) phosphorylation. Both the human and mouse proteins have been previously shown to localize to the nucleolus. The murine protein has also been shown to be highly phosphorylated. To determine whether phosphorylation of treacle is cell cycle dependent, which is one characteristic of nucleolar phosphoproteins, cell synchronization of mouse P19, an embryonic carcinoma cell line, was performed. Using a protocol involving a rapid change in growth in MEMa media, cells were synchronized in G1 phase. A mitotic inhibitor, nocodazole, and mitotic shake off were used to collect cells in mitosis. Synchronization was monitored by flow cytometry. Immunoprecipitation was performed using lysates from synchronized cells. The results were visualized using anti-treacle antibodies after electroblotting on SDS-PAGE and isoelectric focusing gels. The result shows that treacle is hyperphosphorylated in mitosis compared to the protein in G1 phase. Thus treacle has two different forms depending on the degree of phosphorylation. This work confirms that treacle is a member of nucleolar phosphoprotein family and shows that the function of treacle may be regulated by the cell cycle.

Human L1s retrotransposition: cis-preference vs. trans-complementation. *J.V. Moran¹, N. Gilbert¹, J. Boeke², H. Kazazian³, E. Ostertag³, S. Loon², W. Wei¹.* 1) Department of Human Genetics and Internal Medicine, University of Michigan Medical School, Ann Arbor, MI; 2) Department of Molecular Biology and Genetics, Johns Hopkins School of Medicine, Baltimore, MD; 3) Department of Genetics, The University of Pennsylvania Medical School, Philadelphia, PA.

Long Interspersed Repetitive DNA Elements (LINEs or L1s) comprise approximately 17% of human DNA; however, only 60 of the ~400,000 L1s are mobile. Using a L1 retrotransposition assay in cultured human cells, we demonstrate that the L1-encoded proteins predominantly mobilize the RNA that encodes them. This profound cis-preference mechanism allows L1 to remain retrotransposition-competent in the presence of an overwhelming number of non-functional L1s. We further demonstrate that the L1-encoded proteins also can act in trans at much lower levels to promote retrotransposition of mutant L1s and other cellular mRNAs, creating processed pseudogenes. Remarkably, some of the resulting pseudogenes are chimeric; their 5' ends are derived from L1 and their 3' ends are derived from cellular mRNA. Thus, akin to vertebrate retroviruses, some L1 retrotransposition intermediates may contain at least two RNA molecules.

Retroelements in the 5' untranslated region of a human zinc finger gene modulate transcription and translation efficiency. *J.-R. Landry^{1, 2}, P. Medstrand², D.L. Mager^{1, 2}*. 1) Medical Genetics, University of British Columbia, Vancouver, B.C., Canada; 2) Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, B.C., Canada.

A significant proportion of the human genome consists of repetitive sequences. Although long believed to be useless or junk DNA, retroelements, such as Alu sequences, L1s and HERVs (human endogenous retroviruses), have recently been implicated in the regulation of various genes. Our laboratory previously identified a novel, alternatively spliced zinc finger gene, ZNF177, which incorporates Alu, L1 and HERV segments into the 5' untranslated region (UTR) of transcripts. In this study, we investigated the functional significance of those repetitive sequences in the 5' UTR of ZNF177 mRNAs. Using luciferase and GFP reporter constructs, we assessed the effect of the HERV, Alu and L1 sequences on gene expression levels. Our results indicate that the presence of the retroelement sequences, particularly the Alu and L1 segments, in the 5' UTR significantly modifies the expression level of both reporter genes. We present evidence that the Alu and L1 sequences alter both the RNA and protein levels of reporter genes by increasing transcription efficiency while decreasing translation efficiency. Our findings suggest that the Alu and L1 repeats in the 5' UTR of ZNF177 exert a positive transcriptional enhancer effect but repress translation of the zinc finger gene. These results illustrate the complex regulatory effects that retroelements can have on human gene expression.

Comparison of expression and toxicity of long polyglutamine and polyleucine stretches in mammalian cells. *J. Dorsman, B. Pepers, M. Bremmer-Bout, M. IJszenga, G.J.B. Van Ommen, J.T. Den Dunnen.* CBG-Dept Human Genetics, LUMC, Leiden, The Netherlands.

Several neurological disorders are caused by the expansion of a trinucleotide repeat. The largest group of these diseases, including Huntington's disease (HD) and many forms of spinocerebellar ataxia's (SCA) are associated with an expansion of a (CAG)_n repeat in the coding region of the gene. Since the instability of the (CAG)_n repeat lies at the DNA level there is no a priori reason why the sequence (CAG)_n or its complement (CTG)_n would not occasionally be translated as (gln)_n, (leu)_n, (ser)_n, (ala)_n or (cys)_n, depending on reading frame and translated strand. Yet, in the neurodegenerative disorders only gln-repeat expansions are found, while the occurrence of repeats encoding (very) long stretches of other amino acids has not been documented in the sequence databases. To address why this might be so and to determine the potential effect of other repeats, we first needed to overcome the pronounced instability of long homogeneous (CAG)_n repeats in bacterial, yeast and mammalian systems. Therefore, we have constructed a 300-mer of a mixed nonrepetitive DNA oligomer consisting of CAG and CAA triplets. This sequence replicates stably in pro- and eukaryotes and encodes ca 300 glutamines or leucines in two different reading frames. The results obtained in the transfection experiments using cultured mammalian cells indicate that both (gln)₃₀₀ and (leu)₃₀₀ display a high propensity for aggregation. Immune-histochemistry of the transfected cells suggests however that polyleucine is significantly more toxic than polyglutamine, implying that nature may select against reading frames or polarities for (CAG)_n:(CTG)_n repeats other than that coding for polyglutamine. As a follow-up of these studies, we are currently studying the gene expression changes mediated by expression of polyglutamine and polyleucine stretches in mammalian cells using DNA chips and micro-arrays. These studies should provide insight in the cellular changes and the toxic effects of these aggregation-prone proteins.

Development and characterization of a unique set of anti-FMRP antibodies using a novel strategy. *S.S. Ceman*^{1, 2}, *A. Kenneson*^{1,2}, *R. Nelson*^{1,2}, *C. Jin*¹, *L. Lakkis*^{1,2}, *V. Brown*^{1,2}, *K. Wilkinson*¹, *S.T. Warren*^{1,2}. 1) Departments of Biochemistry, Genetics, and Pediatrics, Emory Univ, Atlanta, GA; 2) Howard Hughes Medical Institute, Atlanta, GA.

Full characterization of the function of the FMR protein (FMRP), deficient in fragile X syndrome, has been hampered by a paucity of useful antibodies. In part, this is due to the highly conserved nature of the FMR1 gene throughout vertebrates, which limits its immunoreactivity in animals, as it would appear as a self antigen. To circumvent this problem, we immunized the Fmr1 knockout mouse with recombinant murine Fmrp, as this protein would now be viewed as foreign by the immune system of the mouse. We successfully obtained a collection of monoclonal antibodies (mAbs) that were determined to recognize murine Fmrp by ELISA. By western analysis, we identified mAbs that recognize only mouse Fmrp or both mouse and human FMRP. Importantly, we have identified new mAbs with features not found in the available mAb, 1C3. For example, mAb 7G1-1 found in this screen, unlike 1C3, recognizes only Fmrp and not related proteins Fxr1P and Fxr2P. Indeed, epitope mapping of both 1C3 and 7G1-1 revealed that 1C3 recognizes two epitopes of Fmrp (VEVR and VVRVR) which are both found in the Fxr proteins, while 7G1-1 recognizes the peptide KHLDTKENTHF, which is unique to rodent Fmrp, not being found within the Fxr proteins. Moreover, 7G1-1 and other mAbs developed here have the ability, which was not previously available, to efficiently immunoprecipitate Fmrp. Using these new mAbs we have been able to immunoprecipitate the Fmrp-associated mRNP complex containing associated proteins and mRNAs. Further, because of the specificity of 7G1-1 for Fmrp and not for related family members, we demonstrate conclusively that the existing Fmr1 knockout mouse produces no detectable Fmrp, a point of contention due to the inability of previous antibodies to recognize solely Fmrp.

Huntingtin Interacting Protein 1 Knock-Out Mice are Viable and Provide Insights into the Developmental Expression of HIP1. *M.M. Metzler¹, L. Gan¹, R. Oh¹, M. Gertsenstein², A.S. Hackam¹, A. Borowski¹, F.R. Jirik¹, A. Nagy², M.R. Hayden¹.* 1) Centre for Molecular Medicine and Therapeutics, UBC, Vancouver, Canada; 2) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada.

The neurodegenerative disorder Huntington Disease (HD) is caused by polyglutamine expansion in the ubiquitously expressed protein huntingtin. Altered interaction with brain-predominant proteins, such as huntingtin interacting protein 1 (HIP1), have been proposed as a mechanism for the selective neuronal loss. Decreased interaction between HIP1 and huntingtin following polyglutamine expansion has been observed by yeast two hybrid analyses. This altered interaction may lead to changes in the function of both proteins, thereby contributing to the pathology in HD. Although the function of huntingtin and HIP1, a cytoskeletal-associated protein, is unknown evidence suggests that both proteins are involved in intracellular transport processes. Interestingly, overexpression of HIP1 in 293T and neuronal NT2 cells is toxic and results in caspase 3 activation and subsequent cell death. This observation may be important in understanding the pathologic mechanism in HD since striatal and cortical neurons in HD patients degenerate by apoptosis and high levels of HIP1 protein are expressed in affected brain regions. In order to assess *in vivo* function of HIP1 and its potential role in HD pathogenesis a targeted mutation was introduced into the HIP1 locus by homologous recombination in embryonic stem (ES) cells. A promoter trap targeting vector was used which allows expression of the b-galactosidase gene under control of the endogenous HIP1 promoter. Homozygous HIP1 mutant mice are viable and show no obvious phenotype at 4 weeks of age. Western blot analysis demonstrated that HIP1 expression which is present in frontal cortex and striatum of wild-type mice, has been ablated in HIP1^{-/-} mice. Future studies will determine whether more subtle abnormalities in HIP1 mutant mice occur and whether these mice develop a phenotype at later stages of development. Moreover, HIP1 mutant mice represent an excellent tool to determine the developmental expression of HIP1.

The human *MJD* gene: genomic structure, transcripts, and expression. *Y. Ichikawa*^{1,2}, *J. Goto*^{1,2}, *M. Hattori*³, *A. Toyoda*³, *K. Ishii*³, *S.-Y. Jeong*², *H. Hashida*^{1,2}, *N. Masuda*^{1,2}, *K. Ogata*^{1,2}, *Y. Suzuki*^{1,3}, *S. Sugano*¹, *F. Kasai*¹, *M. Hirai*¹, *P. Maciel*^{4,5}, *G.A. Rouleau*⁵, *Y. Sakaki*^{1,3}, *I. Kanazawa*^{1,2}. 1) Univ of Tokyo, Tokyo, Japan; 2) CREST, Japan Sci and Tech Corp, Saitama Japan; 3) RIKEN Genomic Sci center, Kanagawa, Japan; 4) UnIGENE, IBMC, Porto, Portugal; 5) McGill Univ, Montreal, PQ, Canada.

Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative disorder characterized by cerebellar ataxia and variable associated symptoms. The disease is caused by an unstable expansion of the CAG repeat in MJD that maps to chromosome 14q32.1. The original cDNA clone, MJD1a, consists of 1776bp. We constructed a contig that spanned approximately 300kb, was composed of 6 cosmid clones and 8 BAC clones: B472N19, B472P19, B445M7, B386D20, B400P9, B531L24, B750O6, and B372P2. We sequenced a BAC clone: B445M7 including the *MJD* gene. We analyzed Northern blots and screened four human cDNA libraries and a CAP site cDNA library. Northern blot analysis showed at least four transcripts of which sizes were approximately 1.4, 1.8, 4.5 and 7.5kb, and were ubiquitously expressed. By screening of the four human cDNA libraries of whole brain, caudate, retina, and testis, we obtained 27 clones, including 21 independent clones. Two alternative splicing sites and 8 polyadenylation signals were identified. We determined the complete sequence (175,330bp) of B445M7. These results indicate that the *MJD* gene spans 48,240bp, composed of 11 exons, and coded, at least 5 transcripts, the sizes of which were approximately 1.4, 1.9, 2.0, 4.8 and 7.0 kb, by alternative splicing and polyadenylation.

Spinocerebellar ataxia type 12 (SCA12): Additional evidence for a causative role of the CAG repeat expansion in PPP2R2B. *S.E. Holmes¹, H. Fujigasaki³, E. O'Hearn¹, S.E. Antonarakis⁴, J.K. Cooper¹, C. Callahan¹, J. Hwang¹, D. Gorelick-Feldman¹, I.C. Verma², R. Saxena², A. Dürr³, A. Brice³, C.A. Ross¹, R.L. Margolis¹.* 1) Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Dept of Medical Genetics, Sir Ganga Ram Hospital, New Delhi, India; 3) INSERM U289, Hôpital de la Salpêtrière, Paris, France; 4) University of Geneva Medical School, Geneva, Switzerland.

SCA12 is an autosomal dominant neurodegenerative disorder associated with an expansion of a CAG repeat in the 5'UTR of the gene PPP2R2B, which encodes a brain-specific subunit of the phosphatase PP2. In the originally described family of German origin, the expansion mutation ranged from 66 to 78 triplets. The phenotype typically begins with tremor in the 4th decade, progressing to include ataxia and other cerebellar and cortical signs. We have now identified a second family of different ethnic origin, with a similar phenotype and an expansion ranging from 55 to 61 triplets (Fujigasaki et al., submitted). We have examined the genomic structure of PPP2R2B, and have identified 9 exons which contribute to the known transcript. To better understand the effect of the expansion on the expression of PPP2R2B, we have cloned a 1.7kb fragment of the 5' region of PPP2R2B containing the CAG repeat into reporter constructs. Expression was assayed in the neuroblastoma cell line LA-N-1. The results indicate that this region of PPP2R2B functions as a promoter, comparable in activity to an SV40 control promoter, and that the promoter containing an expanded repeat increases reporter gene expression. We postulate that, unlike other known repeat expansions, the CAG expansion in the 5' region of PPP2R2B increases gene expression.

PHR1, A PH Domain-Containing, Integral Membrane Protein Expressed Predominantly in Primary Sensory

Neurons. *S. Xu*^{1,2}, *R. Ladak*³, *D.A. Swanson*⁴, *R.R. McInnes*³, *D. Valle*². 1) Predoctoral training program, Johns Hopkins Univ, Baltimore, MD; 2) Howard Hughes Medical Institute, Inst Genetic Med, Johns Hopkins University School of Medicine, Baltimore, MD; 3) Programs in Developmental Biology and Genetics, Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada M5G1X8;; 4) Genetics and Molecular Biology Branch National Human Genome Research Institute, Bethesda, MD.

In a differential hybridization screen of an arrayed retinal cDNA library, we cloned a gene, PHR1, expressed at high level in retina. We showed that PHR1 utilizes two promoters and alternative splicing, to produce four isoforms, each with a pleckstrin homology (PH) domain at its N-terminus and a transmembrane domain at its C-terminus. We also showed that PHR1 is an integral membrane protein, abundant in photoreceptor outer segments, pineal body and also in retinal ganglion cells. In in vitro binding assays, PHR1, unlike many other PH domain proteins, does not bind any of a series of inositol phosphates, phosphatidylinositides or other phospholipids. PHR1 does, however, bind transducin $\beta\gamma$ subunits, suggesting a possible interaction with G proteins. To better understand PHR1 function, we made a Phr1 knockout mouse model inserting bgal and neor genes into exon 3 and bringing the bgal gene under the control of Phr1 promoters. By X-gal staining, we found that Phr1 is highly expressed in virtually all primary sensory neurons, including olfactory receptor neurons, taste cells and hair cells in the vestibular and cochlear sensory epithelia of the inner ear. We also found specific expression of Phr1 in certain interdental cells in the spiral limbus of the cochlea, shown recently to have the potential to develop into hair cells. Despite this widespread and specific expression, the Phr1 knockout mice have no discernable phenotype at age 6 months: ERGs, ABRs and rotorod tests are normal as is the histology of the retina, olfactory system and inner ear. Nevertheless, the specific expression of Phr1 in sensory neurons and the potential functions of the PH domain indicates PHR1 may play an important role in the function of sensory neurons.

Studies of X inactivation and the identification of *XIST* in the insectivore *Scapanus orarius*. S. Karalic, C.J. Brown.
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X inactivation is the transcriptional silencing of one of two Xs in mammalian females and is thought to be a means of dosage compensation. The molecular mechanisms of this process are not fully understood; but involve the *XIST* functional RNA that is only expressed from the inactive X, histone hypoacetylation, late replication and hypermethylation. While X inactivation seems to exist in all three mammalian subclasses, there are significant differences regarding the parental origin, stability and extent of inactivation. Delayed replication timing and hypoacetylation have been observed in marsupials, but the roles of hypermethylation and *XIST* may be less conserved. We have examined methylation and *XIST* expression in *Scapanus orarius* (Coast mole), a member of the highly diverged and possibly basal eutherian order Insectivora. Methylation is associated with silencing in eutherians, but apparently not in marsupials. We performed methylation-sensitive restriction digests followed by PCR with conserved primers for two X-linked genes, *ZFX* and *FMRI*. Methylation of the inactive X is conserved in insectivores, as *FMRI* appears to be methylated in females but not males. No methylation is observed for *ZFX*, consistent with it escaping inactivation. *XIST* expression is crucial for initiating inactivation in mice, but has not yet been identified in marsupials, possibly due to low sequence conservation of functional RNAs. Full-length *XIST* sequences are available for human and mouse, as well as small fragments for cow, horse, rabbit, cat and rat. We now report that PCR primers designed from conserved sequence were successful in amplifying 245 bp of Coast mole *XIST* that shows significant similarity (59-84%) to other *XIST* sequences. Furthermore, expression has only been detected in females. The presence of *XIST* in an insectivore indicates that its involvement in X inactivation is conserved within the eutheria. The full sequence of Coast mole *XIST* may highlight conserved functional domains, which could facilitate the identification of a potential marsupial *XIST* and provide valuable information on the evolution and mechanisms of mammalian X inactivation.

***In vivo* footprint analysis of intronic *HPRT* MARs on the active and inactive X chromosomes.** S.-H.L. Kang^{1,2}, C. Chen^{1,2}, T.P. Yang^{1,2,3}. 1) Department of Biochemistry & Molecular Biology; 2) Center for Mammalian Genetics; 3) Department of Pediatrics Division of Genetics, University of Florida, College of Medicine, Gainesville, FL.

Matrix associated regions (MARs) are specific DNA sequences with characteristic AT-richness and topoisomerase II consensus binding sites that bind to the nuclear matrix. Although the role of MARs is not well understood, they are thought to be involved in chromosome organization, DNA replication and/or transcriptional regulation. A role for MARs in X-chromosome inactivation is suggested by the recent observation that the *XIST* RNA is localized to the nuclear matrix. Thus, MARs may mediate the silencing activity of the *XIST* RNA for genes on the inactive X chromosome. We have examined 2 intragenic MARs at the X-linked human *HPRT* locus in introns 1 and 3. DNase I *in vivo* footprint analysis of the 2 intronic MARs was performed to examine sequence-specific DNA-protein interactions on the active and inactive alleles. No DNase I footprints were detected over the MAR in intron 1 on either the active or inactive allele. However, 3 footprinted regions were detected over the MAR in intron 3. All 3 of these footprints are found on both the active and inactive alleles indicating that proteins are bound to this MAR regardless of whether the locus is actively transcribed or silenced. This suggests that the MAR in intron 3 may act as an attachment site of the *HPRT* locus to the nuclear matrix irrespective of transcriptional state and thus plays a role in the global gene organization rather than in transcriptional regulation.

Large-scale gene function discovery in the post-genome era. *A.T. Sands.* President & CEO, Lexicon Genetics Incorporated, The Woodlands, TX.

The dramatic increase in the accumulation of human genomic DNA sequence information has far outpaced the rate at which current technologies enable the discovery of gene function. This gene function gap has significant implications for the development of new therapeutics and diagnostics from the human genome. We have developed a systematic functional genomics methodology to discover the physiologic functions of genes in mammals on a large-scale. Our novel method of high-throughput gene trapping has broad applicability in eukaryotic genomes for gene function discovery. The ability to create mutations in mouse embryonic stem (ES) cells on a large-scale provides a powerful approach for rapidly determining gene function in a mammalian system. Lexicons gene trap technology allows for the automated production of sequence tags from the trapped genes with rapid bioinformatics analysis. We have used these techniques to create the worlds largest library of mouse ES cells called OmniBank that now contains over one-third of genes in the mammalian genome catalogued using bioinformatics in a relational database (www.lexgen.com). We have implemented systems and infrastructure for the generation and analysis of mutant mice at an unprecedented rate of more than twenty novel mutant lines per week. Such a capacity obviously raises the challenge of obtaining meaningful phenotypic information from such mutant lines at a concomitant pace. Fortunately, increasingly widespread recognition of the importance of mouse genetics as a tool in functional genomics has led to the development of mouse scale clinical technologies such as CAT scanning, ECG, CBC and DEXA equipment. Effective application of these technologies now permits the accumulation of a wealth of physiological data on numbers of mutants, which would have been inconceivable as recently as two years ago. Data will be presented on the quality and quantity of information which can now be gathered in screening mode for large numbers of mutant mice in short periods of time. The rapid acquisition of meaningful physiological, structural and behavioral information in mutant mice promises to revolutionize mammalian functional genomics and drug target validation as we enter the post-genome era.

Homozygosity for the connexin 26 167delT mutation in an Ashkenazi Jewish family. *M.Q. Salvador^{1, 2}, M.A. Fox¹, L.A. Schimmenti¹, M. Telatar¹, S. Yazdani¹, W.W. Grody¹.* 1) Departments of Pediatrics, Human Genetics and Pathology, UCLA School of Medicine, Los Angeles, CA; 2) Program in Genetic Counseling, California State University at Northridge, CA.

A 31 year old G2P2 Ashkenazi Jewish woman was referred for genetic counseling for a history of profound nonsyndromic sensorineural deafness believed due to prenatal rubella exposure. Her 33 year old husband, also deaf, believed his deafness resulted from prenatal rubella and x-ray exposure. Their sons, ages 24 months and 10 months, also had sensorineural deafness. The couple sought genetic evaluation to determine why both of their sons were deaf. The 24 month old son had been diagnosed at 19 months with a 90 decibel loss in the right ear and a 40-80 decibel loss in the left. He had normal tympanograms and a normal CT scan of the temporal bones. The 10 month old son was diagnosed at 5 months by audiogram to have a 70-90 decibel loss in the right ear and 90 decibel loss in the left ear with normal tympanograms. The family reported that the 10 month old appeared to be hearing in the newborn period, but brainstem auditory evoked response studies were unavailable. Mutation analysis of the connexin 26 GJB2 gene was performed by PCR and allele-specific restriction endonuclease digestion on genomic DNA from the parents and both sons. All four members of the family were shown to be homozygous for the 167delT mutation, which has a reported carrier frequency of 4% in the Ashkenazi Jewish population. The findings in this family illustrate the importance of considering mutations in connexin 26 as a significant cause of heritable deafness even when the typical clinical presentation and autosomal recessive inheritance pattern are not apparent. Given that approximately 50% of nonsyndromic autosomal recessive congenital hearing loss is caused by mutations in the connexin 26 gene, DNA testing will assume a key ancillary role in newborn hearing screening programs. Our experience would suggest that consideration should be given as to whether screening for the common connexin 26 mutations should be incorporated as a first or second tier procedure in early screening for congenital deafness.

A population-based study of knowledge, risk perception, and screening practices among subjects with familial colorectal cancer in Ontario. *O. Ginsburg, M. Aronson, M. Cotterchio, H. Sutherland, S. Gallinger.* The Ontario Familial Colon Cancer Registry, Toronto, Canada.

Objectives: To study risk perception, knowledge, and screening practices reported by CRC (colorectal cancer) patients in the Ontario Familial Colon Cancer Registry (OFCCR). Methods: A questionnaire was administered by telephone to subjects with CRC in the OFCCR, a population-based registry of CRC in Ontario. Results: 119 questionnaires were completed, including subjects from 52 high-risk families (history suggestive of HNPCC), 24 intermediate-risk and 36 low-risk families (sporadic cases randomly sampled from the registry). CRC was detected by screening in 9 people. Although there was no difference in screening patterns between risk groups, high-risk subjects who believed they were at increased risk prior to their diagnosis were more likely to have been screened vs. those who did not think they were at increased risk (28.57% vs. 4.35%, $p = 0.007$). Although 95% of subjects knew at least one symptom of CRC, most could only name rectal bleeding. Only 42% named change in bowel habit as a symptom. Few knew of constitutional symptoms: fatigue (16.1%), and weight loss (10.1%). High-risk subjects were no better than low-risk at naming symptoms. Subjects whose families spoke openly about cancer prior to their diagnosis were slightly more likely to be diagnosed by screening vs. subjects who did not (77.8% vs. 55.96%, $p = 0.20$). 80.6% of the patients referred for genetic counseling received counseling by the study completion. Of persons who did not speak openly prior to their diagnosis but received subsequent genetic counseling, all stated they speak openly now vs. only half of those who did not receive counseling (100% vs. 50%, $p = 0.001$). Conclusions: Although much has been published on hereditary CRC, our results indicate that few cases are detected by screening, even in high-risk families. To our knowledge this is the first North American study of attitudes, risk perception, and screening from a population-based registry of CRC. Further studies are needed to address the apparent lack of awareness about familial CRC syndromes among high-risk families and their health care providers.

Psychosocial issues following a positive genetic test for BRCA1 and BRCA2: Findings from a focus group and a needs assessment survey. *J. Honeyford*¹, *L. Di Prospero*², *M. Seminsky*², *B. Doan*², *E. Franssen*², *P. Chart*², *E. Warner*², *W. Meschino*^{1,2}. 1) Genetics, North York General Hospital, Toronto, ON, Canada; 2) Toronto-Sunnybrook Regional Cancer Centre, Toronto, ON, Canada.

Genetic testing for BRCA1 and BRCA2 has been available in Ontario for the past 5 years on a research basis. The purpose of our study was to obtain feedback about how genetic testing had affected our patients and their families, and to explore whether there was interest or need for ongoing support beyond routine follow-up in the Familial Breast Cancer Clinic.

Twenty-seven BRCA1 and BRCA2 mutation carriers who had received genetic test results at either of two Familial Breast Cancer Clinics were invited to participate in a focus group and concurrently complete a questionnaire (participants), with 8 agreeing to attend the group (30%). A slightly revised version of the questionnaire was subsequently mailed to 26 mutation carriers who did not participate in the focus group (respondents). Of these, 16 were completed and returned (62%).

The questionnaire was divided into 9 main areas of interest: 1) demographic information, 2) the effect of genetic testing on cancer risk perception, 3) the effect of genetic testing on worry about cancer, 4) communication with family members since receiving test results, 5) attitudes towards surveillance, 6) attitudes towards preventive options, including prophylactic surgery, chemoprevention, diet and/or lifestyle changes, 7) satisfaction with clinical services, 8) need for additional support, and for non-focus group participants 9) satisfaction with their decision to have undergone genetic testing.

Results suggest that risk perception and cancer worry increase after carriers receive test results. Although all participants and respondents were highly satisfied with clinical services provided to them, a significant proportion of BRCA1 and BRCA2 mutation carriers strongly favoured the availability of support groups. Organization of such support groups should be a priority for clinical cancer genetics programs.

ATTITUDE TOWARDS PROPHYLACTIC SURGERY AND EFFECTS OF GENETIC COUNSELLING IN FAMILIES WITH BRCA MUTATIONS. *R. Moeslinger¹, T. Wagner¹, G. Langbauer¹, Austrian Hereditary Breast and Ovarian Cancer Group¹, E. Fleischmann¹, A. Autherith², A. Friedmann³, T. Helbich⁴, P. Oefner⁵.* 1) Dept OB/GYN, Div Senology, Vienna, Austria; 2) Department of Statistics, University of Vienna; 3) Department of Psychiatry, University of Vienna; 4) Department of Radiology, University of Vienna; 5) Stanford DNA Sequencing and Technology Center.

The intent of this study was to evaluate the effect that awareness of being a BRCA1 or BRCA2 mutation carrier has on the attitude towards prophylactic surgery and on developing depression symptoms,. Sixty three families were selected on the basis of previously detected BRCA1 or 2 mutations and 142 family members were given the appropriate questionnaires. Prophylactic mastectomy (PM) was considered by 23% of the Austrian mutation carriers (28% affected and 17% non-affected carriers). The majority of affected and non-affected carriers expected PM to impair the quality of their life. Sixty percent would undergo prophylactic oophorectomy (68% affected and 50% non-affected carriers). The self-rating depression scale indicated that following mutation result disclosure the depression scores of carriers decreased (40 baseline vs. 39 after result disclosure, $p=0.5$), whereas, for non-carriers, scores increased (37 baseline vs. 39 after result disclosure, $p=0.8$). However in affected carriers only 4% have performed PM respectively only 17% PO and in non-affected carriers these percentages were even lower: Only 2% performed PM and 13% PO. We conclude, that information about carrier status is not associated with increased depression symptoms in mutation carriers. In non-carriers depression scores increased slightly, probably reflecting survivor guilt. The option of having PM was associated with a negative impact on the quality of life and was declined by the majority of Austrian mutation carriers.

Counseling of Parents of the Neonate with Down Syndrome: The Need for Echocardiography. *E.H. Zackai¹, M. Straka¹, E. Goldmuntz²*. 1) Division of Human Genetics and Molecular Biology; 2) Division of Cardiology, The Children's Hospital of Philadelphia, Philadelphia, PA.

The counseling of the parents of a newborn with Down syndrome is multifaceted and since the incidence of congenital heart disease is high, it is important to determine the minimal screening procedures sufficient to identify the congenital heart defects. We undertook a study to determine whether normal cardiac exam was predictive of normal cardiac anatomy. 118 Down syndrome infants, ages birth-30 days, had a cardiac exam and subsequent echocardiogram regardless of initial findings. Of the 66 who had an abnormal cardiac physical exam, 55 (83%) had significant intracardiac defects (VSD - ventricular septal defect, CAVC - common atrioventricular canal, TOF - tetralogy of Fallot), while 8 (12%) had a PDA (patent ductus arteriosus) only and 3 (5%) had normal anatomy by echocardiogram. 52 patients had a normal cardiac physical exam, of which 19 (36.5%) had intracardiac anomalies (VSD, CAVC, bicuspid aortic valve), 14 (27%) had PDA only, and 19 (36.5%) had normal cardiac anatomy by echocardiogram. None of the 15 patients with a TOF had a normal cardiac exam, however, 10 of 37 patients with CAVC and 8 of 21 patients with VSD had a normal neonatal cardiac exam. Thus, the cardiac exam alone would have been falsely reassuring 19 out of 52 cases (36.5%) and these patients would most likely present later with a murmur or symptoms of congestive heart failure. The sensitivity of the clinical cardiac exam was only 74% (55/74). Thus, performing an echocardiogram on neonates with Down syndrome regardless of normal clinical cardiac exam, would increase the sensitivity of detection of the heart defect to 100%. It is noted that a cohort of patients with normal cardiac exam underwent echocardiogram and were anatomically normal as well (19/52; 36.5%) and that patients diagnosed with a PDA in the neonatal period (possibly representing normal transitional circulation) will require re-evaluation. Ultimately, however, echocardiogram in the neonate with Down syndrome leading to definitive diagnosis, either allays parental concerns or permits parental adjustments and allows for early presymptomatic care.

Current views on continuing education and employee awareness assessed by a voluntary questionnaire during The 6th Annual New England Regional Genetics Conference: A need assessment for improved educational forums for genetic laboratory personnel. *J.M. Luszcz, R. Naeem.* Dept. of Pathology, Baystate Medical Center, The Western Campus of Tufts University School of Medicine, Springfield, MA.

Recent advances in the human genome project have given rise to rapid developments in genetics. As a result, the provision of continuing education for those who work in the field is paramount in maintaining and improving both awareness and practical skills. A voluntary questionnaire was employed during The 6th Annual New England Genetics Conference to ascertain the opinion of genetic laboratory personnel regarding job satisfaction, laboratory competence, and their employers focus on continuing education. Forty one questionnaires were completed by attendees and subsequently reviewed. Approximately one third felt they were undereducated for their current duties. Interestingly, 85% had stated they had attended an educational event within the last year. Forty six percent felt they had reached a educational or training plateau at their current position. In response, 95% stated they would attend more educational events if made accessible by the employer. When asked about educational benefits, journals, regional genetics seminars, and local specialist lectures were rated in the fiftieth percentile. Sixty six percent said they had not attended a national genetics conference due to either financial difficulty, lack of support or the inability to receive time off. Fifty seven percent stated they were unsatisfied with their current salary status. In addition, 69% stated they would consider leaving the field of genetics for a better paying opportunity in an alternative career. Furthermore, only 29% felt they would still be working in laboratory genetics within the next five years. Based on this information it appears that an effort to improve continuing education policies in the workplace is warranted. Discrepancies with salaries, responsibilities, and employee support need further investigation in order to maximize productivity and minimize turnover rates in this particular subset of the field. In addition, a national survey is currently underway to determine similar trends across a broad geographical base.

First, the bad news: What people with Marfan syndrome(MS)find when they search the internet for information.

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As physicians feel the impact of managed care, leaving them with less time to spend with patients (pts), the internet is increasingly becoming a primary source of medical information for newly-diagnosed individuals. Because no editorial control exists, there is the potential that pts may encounter false information, raising the potential for harm. To investigate this, we examined information available on "Marfan-list" (www.widowmaker.com/~jnavia/list.htm), a pt-run website consisting of a list of "subscriber" entries, and compared the clinical manifestations of MS reported by pts to those reported in a large retrospective series (Sun et al., Am Heart J, 120:934-948, 1990)

Of the 206 entries listed on "Marfan-list", 115 (56%) were written by pts and described clinical manifestations in enough detail to be analyzed. The website population reported a much higher incidence of serious cardiac manifestations (79% vs 38%), and a much lower incidence of ophthalmologic (42% vs 68%) and skeletal manifestations (50% vs 97%) than was observed in the Sun et al. series, indication that more life-threatening (and more fear-inducing) features are over-represented on the website, while the milder features were underrepresented.

Although more work is needed, this preliminary study confirms that information available from at least one pt-run website is skewed to provide more dire features. Physicians should provide guidance to their pts before they begin to search the internet.

An Evaluation of the Usability of Pedigrees Produced by Patient-Driven Software for Stratifying Cancer Risk.

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The ability to determine the degree of hereditary cancer risk has important implications for medical management of the entire family. The best method of assessment of hereditary risk is through examination of the family medical history or pedigree. The process of collecting the pedigree is time-consuming and often necessitates a separate genetic counseling visit. In order to streamline the process of risk assessment we have developed a web-based pedigree-builder system. In this study we compared the web-based pedigree with a pedigree produced by a genetic counselor for accuracy, completeness, and performance during cancer risk assessment. Pilot data with 12 patients indicates that using the web-based tool is acceptable to patients and can be completed without assistance. We collected pedigrees from forty patients recruited from a community-based oncology office. Participants provided their pedigree both by meeting with a genetic counselor and by recording their pedigree using the web-based software. The study included a crossover design in which half of the patients met first with a genetic counselor, and half used the web-based pedigree-builder system first. Using the counselor pedigree as the gold standard, the web-based pedigree was graded for accuracy and completeness. Then the pedigrees were analyzed using expert reviewed, rule-based computer algorithms designed to stratify their degree of hereditary cancer risk (general population, elevated risk, markedly elevated risk, or meeting established criteria for a hereditary syndrome). Results will be presented comparing the on-line tool with in-person collection methods with regard to: pedigree accuracy and completeness, and concordance for cancer risk stratification.

The development of algorithms to assess family health history in a web based application - HealthHeritage. *S.L. Pelletier¹, B.B. Worrall², M.B. Harrison², S. Miesfeldt³, A.L. Tucker³, T.E. Kelly⁴, P. Schnatterly⁴, J. Einbinder¹, M. Reitmeyer³, W.F. Cohn¹, W.A. Knaus¹.* 1) Department of Health Evaluation Sciences, University of Virginia Health System, Charlottesville, VA; 2) Department of Neurology; 3) Department of Internal Medicine; 4) Department of Pediatrics.

Advances in the understanding of the genetic basis of common diseases, including those that are multifactorial, have the potential to change the practice of clinical medicine. Complete patient care may involve the ability of the primary care physician to assess genetic risk and make individual patient recommendations regarding prevention, screening and treatment. A secure patient-driven web tool has been developed to facilitate the collection and interpretation of family health history information. This tool includes algorithms for assessing individual risk based on family health history in five disease areas: (1) oncology (e.g. breast, ovarian, colon, prostate, kidney, skin, thyroid); (2) cardiology (e.g. cardiomyopathy, arrhythmia, structural heart abnormalities); (3) vascular disease (e.g. hypertension, atherosclerosis, metabolic lipid disorders, stroke); (4) neurology (e.g. neuromuscular disease, movement disorders, epilepsy, dementia); and (5) endocrinology (e.g. diabetes, osteoporosis). Risk algorithms were developed using comprehensive, evidence-based literature reviews and consultation with genetic specialists. Rules were developed to stratify risk for autosomal dominant, recessive, mitochondrial and X-linked conditions. Factors that modify risk were included in development of algorithms for the multifactorial conditions. These algorithms make the process of risk assessment explicit and can be updated if new risk information becomes available. Management recommendations are based on the best available evidence, published guidelines and expert opinion and include screening, treatment, prevention and risk modification. The tool has the potential to increase recognition of the influence of genetics on disease in the primary setting and decrease the burden on primary care physicians. A pilot test of HealthHeritage in the primary care setting is currently underway.

Views and attitudes toward malformations among nursing college students. *H. Kawame*^{1,3}, *Y. Fukushima*², *Y. Eto*³.
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In Japan, an increasing percentage of hospitalized children have congenital malformations. Factors contributing to this increase include improved medical management, improved clinical genetics services, and longer length of stay for medical and social reasons. Currently, genetic counseling, as a profession, does not exist in Japan. Therefore nurses provide much of the genetic counseling, which these children's families receive. This pilot study was designed to characterize the attitudes of Japanese nursing students toward visible malformations. Information from this study can be used to develop genetic curricula for these students. A questionnaire was given to 61 3rd year nursing students at a private medical university in Tokyo. Fifty eight percent of the students previously had contact with individuals with physical malformations. All 61 students reported a personal negative attitude toward physical malformations, especially to the Japanese word "ki-kei" (strange shape). They also all felt that current Japanese society would respond negatively to people with physical malformations. The majority (74%) thought of malformations as a "disease" and considered people with malformations as being "disabled" or "handicapped." On the other hand, 75% considered the malformation to be only one aspect of the child's personality. Regarding prenatal diagnosis, 45% felt that they would consider prenatal diagnosis for malformations and 38% would not. Almost half (49%) said that prenatal diagnosis of an abnormality would inevitably lead to a termination. In an open-ended question about what professionals can do for families with malformations, the nursing students suggested providing as much information as possible, referring families to support groups, helping mothers deal with feelings of guilt, and providing a non-discriminatory atmosphere for families. We conclude that genetic training programs for nurses should include early and frequent exposure to various disabilities and more formal education on congenital malformations and genetic disabilities.

Parental Attitudes Toward Genetic Testing for Pediatric Deafness. *J.W. Brunger¹, A.L. Matthews¹, M. O'Riordan², G.S. Murray³, R.J.H. Smith⁴, N.H. Robin^{1,2,3}.* 1) Genetics; 2) Pediatrics; 3) Otolaryngology, Case Western Reserve Univ, Cleveland, OH; 4) Otolaryngology, Univ Iowa.

Recent advances in understanding the molecular genetic basis of deafness has resulted in genetic testing becoming an option for deaf individuals and their families. However, there is little information about the level of interest in such testing. To investigate this question, a survey was sent addressing attitudes toward diagnostic, carrier, and prenatal genetic testing for deafness. The target population, parents with normal hearing who have one or more deaf children, represents the vast majority of individuals who will be encountered in clinical practice, as 90-95% of deaf individuals are born to normal hearing persons. Collaborating audiologists, otolaryngologists, and geneticists distributed 328 surveys, and 96 (29%) were completed and returned. Overall, 96% recorded a positive attitude toward genetic testing for deafness, including 70/96 (76%) who were interested in or had had such testing. 61/70 (87%) believed that genetic testing for deafness should be offered prenatally, but only 1/61 stated she would terminate her pregnancy because of profound deafness. The vast majority would not terminate such a pregnancy and did not believe that deafness implied a poor quality of life. In general, however, these parents had a poor understanding of genetics. The vast majority (98%) incorrectly estimated the recurrence risk of deafness, and misunderstood the mode of inheritance in their family. Notably, these findings were similar in both the group who had had genetic testing for their children and those who had not had testing. This similarity suggests that the parents who received genetic testing (n=32) did not receive genetic counseling, or that the counseling was not effective. Based on these results, we conclude that this population is interested in utilizing genetic testing. The testing should not be done without first providing formal genetic counseling. By including appropriate counseling, the risks, benefits, and limitations of genetic testing can be addressed. Each individual then will have the ability to make an informed decision whether to utilize this testing.

Assessment of knowledge regarding the prevention of alcohol related birth defects (ARBDs) and neural tube defects (NTDs) among postpartum women in Washington (WA) State. *M.A. Eaglin, T. Bell, R.M. Fineman.* MCH, Washington State DOH, Olympia, WA.

The relationships between the consumption of alcohol and ARBDs, and the consumption of folic acid (FA) and the prevention of NTDs, are well established. An important question that remains is whether all pregnant women are equally informed about these important health care issues. In order to help address this question, we analyzed self-reported, postpartum (PP) data from the WA State Pregnancy Risk Assessment Monitoring System from April 1996 through December 1998. PP, resident women were asked whether a doctor, nurse, or other prenatal care provider (PCP) talked to them about how drinking during pregnancy could affect their baby, and whether they had ever heard or read that taking the vitamin FA could help prevent some birth defects (BDs). Overall, 81.7% of PP women reported that a PCP talked to them about how drinking during pregnancy could affect their baby; while 70.4% reported hearing or reading about how taking FA could prevent some BDs. This means that, overall, PCPs in WA probably missed a significant number of opportunities to educate pregnant women about the prevention of ARBDs and NTDs. In addition, PCPs were: 1) significantly LESS likely to discuss the effects of drinking during pregnancy with women who were: older than 20, had more than 12 years of education, married, White, and not Medicaid recipients; and 2) MORE likely to discuss the effects of drinking with their traditionally under-served patients. Not surprisingly, maternal attributes associated with NOT having heard or read about the beneficial effects of FA included: maternal age less than 20, education less than 12 years, Hispanic, unmarried at the time of birth, used Medicaid to subsidize prenatal care, and had an unintended pregnancy (i.e., traditionally underserved patients). In order to improve birth outcome, PCPs, public health professionals and others should attempt to educate all women of reproductive age (and especially pregnant women) about these important health care issues, instead of targeting certain groups based on certain socio-demographic attributes (see also Comm Genet 2:61-68, 1999).

Four Years of Experience in a High-Risk Cancer Genetics Clinic: Lessons Learned. *J.P. Evans, C. Skrzynia, E.M. Rholfs, J.K. Booker, L.M. Silverman, Q. Yang, H. O'Lear, M.L. Graham.* Dept Medicine, Univ N Carolina, Chapel Hill, Chapel Hill, NC.

Since 1996 The UNC Cancer Genetics Program has counseled high-risk Breast and Ovarian cancer families and offered BRCA1/2 testing. Over 400 families of diverse ethnicity were counseled. Individuals from 117 families(25%) chose to pursue genetic testing. The UNC Clinical Laboratory performed Protein Truncation analysis (PT) for BRCA1 and 2 and complete sequencing of the BRCA1 coding region. PT variants were confirmed by sequencing. Sixteen different BRCA1 or 2 mutations were found in 17 families, for a mutation incidence of 17%. A total of 50 at-risk relatives were also tested and 45% carried the familial mutation. The presence of phenocopies is a potentially confounding issue in high-risk families. This concern is highlighted by our experience in which 4 individuals who belonged to mutation positive families and had previously developed breast cancer were found to be mutation negative. It has been postulated that African Americans (AA) with a family history of breast cancer are at lower risk of carrying a mutation in BRCA1/2. Of 9 AA families tested, 2 carried a BRCA1 mutation (22%), similar to that of Caucasians. A strong predictor of discovering a familial mutation included the presence of an individual with both breast and ovarian cancer (5/12 or 42% of such pedigrees were mutation positive). Likewise, the presence of breast cancer diagnosed at less than 41yo in a first degree relative indicated a high likelihood of finding a mutation (8/31 or 26%). A variety of statistical models have been developed to predict the chance of finding a BRCA1/2 mutation as a function of family history. The most recent version of BRCAPro (a modeling program) was used to estimate the chance that a mutation would be discovered. For the BRCA1/2 positive individuals, the BRCAPro estimate ranged from 2.9% to 100%, emphasizing the need for clinical judgement in determining appropriate candidates for testing and not exclusive reliance on a statistical modeling program. The above issues should be considered when counseling individuals regarding the decision to undergo genetic testing.

An analysis of reproductive choices within families affected by Huntington's Disease. *B.J. Beyer, V.E. Headings.* Genetics and Human Genetics, Howard University, Washington, DC.

Huntington's disease (HD) is a late-onset progressive neuropsychiatric disorder. Children of affected parents are at 50% risk to develop the disease. Presymptomatic gene testing is now available to determine if at-risk individuals will develop HD. One common reason individuals undergo predictive testing is to make informed decisions regarding reproduction. Therefore, this study examined how individuals in families affected by HD approach the available reproductive options. There were 45 questionnaires completed, with 69% of the participants at-risk to develop HD, and the remaining 31% spouses of at-risk individuals. The reproductive choice considered most likely was to refrain from having children (47.5%), with sperm donation the least likely (87.5%). Males were more likely than females to choose to have children without assisted reproduction ($p=.001$). Spouses were more likely than at risk-individuals to choose sterilization of the at-risk parent ($p=.04$). Those participants less than the median 45 years of age were more likely to choose preimplantation genetic diagnosis than those who were older ($p=.03$). Individuals who learned of HD in their family at a younger age (< 29 years) were more likely to adopt/foster children than those who learned later in life ($p=.042$). None of the participants had undergone prenatal diagnosis in the past. However, 40% would choose prenatal diagnosis in the future, with 38% stating they would terminate a pregnancy with HD. Only 44.8% of the at-risk individuals met with a genetic counselor, and only 13.8% discussed reproductive options during the encounter. However, 25% stated that the appointment changed their opinions regarding reproduction. Therefore, this study strongly suggests that some individuals within families affected by HD have considered various reproductive options, and may benefit from more discussion with genetic counselors. Hence, we feel that the results from this study indicate the necessity of a pamphlet listing and defining the various reproductive options, in addition to identifying diagnostic and counseling services.

Initial phone disclosure of BRCA1/2 results is acceptable in high risk women. *J.R. Klemp, C.J. Fabian.* Breast CA Prev Ctr, Univ Kansas Medical Ctr, Kansas City, KS.

Women at high risk for breast cancer based on family history, precancerous breast disease, and/or prior breast cancer, were eligible to undergo free and confidential BRCA1/2 full sequencing (Myriad Genetic Laboratories, Inc) as part of a 6-month Phase II chemoprevention trial. Women received pre-test counseling performed by a risk counselor at their 4-month visit and prior to drawing their blood sample at their 6-month study completion visit. Ninety-seven percent (116/119) of the eligible subjects underwent testing. In >90% of the subjects, results were initially discussed by phone with either the physician and/or risk counselor. A subsequent in-person consultation was mandatory for uncertain or deleterious subjects including the patient, physician, cancer risk counselor, and additional family members if desired and optional for negative individuals. Subsequently, subjects were sent a satisfaction survey in the mail, 91% (105/116) responded. Twenty-four of the 26 women with a deleterious (3), uncertain mutation (17), or polymorphism (4) responded. One subject with a positive result felt that the information provided needed to be easier to understand and another subject with a deleterious mutation felt that the results should have been disclosed over the phone instead of requiring an in-person visit. Subjects with negative results were satisfied with the testing process. 100% of the respondents felt they made a wise decision in having the testing and 97% (102) would recommend that other woman in a similar situation undergo genetic testing. Access to genetic testing was influential in recruiting eligible women onto the chemoprevention trial: 41% (43) stated access to genetic testing was influential, 43% (45) stated they will be more likely to participate in future trials, and only 3% (3) stated they will be less likely. The vast majority of high risk women participating in a clinical trial will undergo anonymous and free BRCA1/2 genetic testing, were accepting of receiving results initially by phone, and felt they made a wise decision to undergo genetic testing.

Amniocentesis rates in women of Puerto Rican ethnicity. *E.L Habecker¹, J.G Habecker-Green¹, R. Naeem², G.M Cohn¹*. 1) Clinical Genetics, Baystate Medical Center, Springfield, MA; 2) Cytogenetics, Baystate Medical Center, Springfield, MA.

Several studies have been published on the selective use of amniocentesis by patients of different socioeconomic status, educational background, and ethnicity. We were unaware of any studies that specifically addressed uptake of amniocentesis by patients of Puerto Rican ethnicity. We hypothesized that, based on religious and cultural values Puerto Rican patients may opt for fewer amniocenteses than patients of other ethnicities. A retrospective chart review was undertaken of a random sample of patients seen by genetics services at Baystate Medical Center. Patients were selected for analysis if they had been counseled about amniocentesis between 1994 and 1997, and if they had been referred for genetic counseling by Baystates resident clinic. Resident clinic patients were selected in an attempt to control for socioeconomic status and educational background. 200 charts were selected and the following data points recorded: consult date, counselor, patient age, patient ethnicity, whether the patient was accompanied to the consult, the indication for offering amniocentesis, the numeric risk quoted for the relevant anomaly, whether amniocentesis was accepted, and the result of amniocentesis if performed.

Of the 200 charts reviewed, 10 were excluded from further analysis as the ethnicity was mixed Puerto Rican and other or because the ethnicity had been recorded as hispanic. Of the remaining charts, 93 patients were Puerto Rican and 97 were of other ethnicities. Each group was equally distributed with respect to consult date, counselor, whether accompanied, indication, and numeric risk for anomaly. The patients of Puerto Rican ethnicity had an average age of 25.7 years, those of other ethnicities of 27.1 years. 3 chromosomal anomalies / variants were seen, all in patients of Puerto Rican ethnicity. 27% of each group accepted amniocentesis no differences based on ethnicity were seen. This finding would indicate our hypothesis that Puerto Rican patients would choose fewer amniocenteses was incorrect.

The Belief of the Role of Genetics in the Development of Alcoholism. *P. Manowitz¹, R.M. Hamer¹, T.M. Creeden², J. Ballou³*. 1) Dept Psychiatry, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ; 2) Reproductive and Perinatal Genetics, St. Peter's University Hospital, New Brunswick, NJ; 3) Eagleton Institute, Rutgers, The State University of New Jersey, New Brunswick, NJ.

Objective: A number of genetic studies have been conducted to identify genes predisposing to alcoholism. The reason for identification of these genes is that this information may be useful in alcoholism prevention and treatment. However, little if anything is known about the receptivity of the general public to the concept that alcoholism has a genetic basis. **The purpose of this study was to assess the belief regarding the role genetics plays in the development of alcoholism.** **Method:** A general telephone survey was conducted of 953 individuals asking them to assess the effect the following have on a person's likelihood to become an alcoholic: parent's drinking habits when a child is growing up, a bad home situation, genetics - which is a person's genes or biological make-up, or a bad work or job situation. **Results:** Seventy seven percent of the respondents said that genetics has either a lot or some effect on a person's likelihood to become alcoholic. **Conclusions:** For each subgroup examined with regard to a wide variety of variables (age, education, alcohol intake, etc.), the majority stated that genetics has a lot or some effect on alcoholism formation. The data do support the hypothesis that having a parent with an alcohol problem is related to an increased belief in the importance of genetics in alcoholism formation. Even though modifying factors such as age, sex, alcohol intake, and educational level may be operable in determining the extent of the belief, these effects appear to be modest. This information may be helpful in devising future strategies for utilizing genetic information to prevent and treat alcoholism.

Genetic testing for Alzheimer's disease: Preliminary results of a protocol for presymptomatic APOE genotyping.

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Consensus statements have advised against clinical use of APOE genotyping for predictive purposes. Future use of genetic tests to identify patients at an increased risk for AD is anticipated and therefore, an AD susceptibility genetic counseling model needs to be developed. We have conducted a pilot study for APOE genetic counseling and testing. The study protocol consisted of an informational session, genetic counseling and testing, results disclosure, and a follow-up interview. Participants were self-referred from the community. Exclusion criteria included significant neurologic or psychiatric history and cognitive deficits on neuropsychological testing. Subjects completed a questionnaire on genetics and AD before and after the informational session, after the second session, and 3 to 6 months after disclosure of genotype. Thirty-four individuals enrolled in the study and attended the informational session. Seventeen of those individuals consented to APOE genotyping. The primary reasons for wanting testing were future planning (71%), potential for early treatment (57%), and reducing anxiety (48%). The most common reasons for declining testing were fear of insurance discrimination (75%) and lack of treatment for AD (75%). The results indicate that the informational session was effective in conveying factual information. Four individuals possessed at least 1 $\epsilon 4$ allele, and only one of these subjects reported feeling greater anxiety after learning their genotype. Results from the follow-up survey show that counseling had a positive impact and increased most subjects' knowledge about AD genetics. In addition, our results indicate that sense of personal risk decreased over the course of the study for the majority of participants. This protocol can be used as a prototype for future AD susceptibility testing and is undergoing further development as part of an ELSI sponsored study now underway (The REVEAL Study).

Knowledge and Attitudes of Obstetricians and Gynaecologists and Patients towards the Maternal Serum Triple

Screen. *T. Stermer*^{1, 2}, *P. Craig*^{1, 2}, *D. Viljoen*^{1, 2}, *A. Krause*^{1, 2}. 1) Human Genetics, South African Inst Med Res, Johannesburg, Gauteng, South Africa; 2) University of Witwatersrand, Johannesburg, Gauteng, South Africa.

Genetic counselling experiences in Gauteng, South Africa, of patients who have undergone the maternal serum screen (MSS) have indicated that women may not be given sufficient information about the test, resulting in much anxiety. A study evaluating the knowledge and attitudes of Obstetricians and Gynaecologists (O&Gs) and patients towards the MSS was conducted. A questionnaire exploring these subjects as well as the practices of O&Gs with this test was posted to 275 O&Gs in and around the Gauteng region. In addition, patients who received either a high risk/low risk result in the MSS have also been interviewed to determine their attitudes and experiences with this test. So far, 60 questionnaires have been returned (22% return rate) and more are expected. Thirty-five interviews of the 60 interviews targeted have been conducted. Results suggest that O&Gs' factual knowledge concerning this test is relatively good (scoring over 75%), however, in some cases extremely poor (as low as 36%). A large number of doctors (78%) offer this test to all their pregnant patients, and not only to women under 35 years of age, for whom the test is designed, thus resulting in many false positive results. Most O&Gs felt it is unnecessary to refer a patient to a genetic counsellor (57% of the time). Almost all O&Gs felt this test is inadequate in sensitivity and specificity resulting in much unwarranted anxiety, however, as no definite alternative is available, it is still a useful procedure. Patient interviews suggest that although women are aware of the purpose of the test, they are not made aware of its accuracy and sensitivity, and are often falsely reassured with a low risk result. There seems to be a general consensus that receiving an information pamphlet explaining this test, its short-comings and consequences, in a visit prior to the 16-week visit when the test is performed, would be very helpful in making an informed choice whether to have the test or not, based on their personal and religious beliefs.

Monsters, Myths, and Eugenics: Public Perception of the Human Genome Project. *M.Z. Peliás, N.J. Markward.*
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Recent events in the world of gene therapy have attracted close scrutiny to the activities of professionals in medical genetics and genetics research. While the genetics community has celebrated the successful treatment of children in France for SCIDS and grieved the death of a young American man with OTC deficiency, individual commentators and the media have continued to suggest that professional geneticists are less than ethical in the practice of medical genetics and in biomedical research. One report, now 10 years old, began with a long discussion of the monsters of literary fiction, followed by a lengthy examination of the perils of human genome research and the eugenic overtones of medical genetics and genetic counseling. These themes continue to surface, always without thorough explanation of the perceived connections to genetics, and often without definitions and distinctions among similar words. Further, the media, in search of news items, are necessarily constrained by column space and time clips from presenting thorough, comprehensible reports about current activities in medical genetics and genetics research. These approaches to reporting news about genetics nourish fear and suspicion among the lay public and generate one of two responses within the genetics community. Some geneticists, faced with unfounded allegations and generalizations, retreat from public view and, probably unwittingly, contribute to the shroud of mystery that covers research and practice in genetics. Others, at least in small numbers, are available for comment and clarification, and participate in public programs that seek to make genetics and the benefits of genetics research understandable and attractive to the lay public. A forthright approach in public communication should become the standard of conduct for genetics professionals.

Genetic knowledge , opinions and practices amongst general practitioners in South Africa. *K.L.M. Trenton^{1,2}, P. Craig^{1,2}, D. Viljoen^{1,2}, A. Krause^{1,2}.* 1) Human Genetics, South African Inst Med Res, Johannesburg, Gauteng, South Africa; 2) University of the Witwatersrand, Johannesburg, Gauteng, South Africa.

The arena of Human Genetics is playing a greater role in healthcare than ever before. The question arises whether these scientific advances are moving swiftly enough from the field of science to the practitioners of medicine. In general, since the onset of genetic services in South Africa (SA) in 1971, knowledge and attitudes regarding the utilization of these services have often been misguided. Medical institutions and practicing doctors are unaware of the important intricacies of genetics that impact on their patients. Genetic counseling (GC) is underutilized and many genetic conditions are neither recognised nor diagnosed. The field of Human Genetics is clearly beneficial but in SA has lagged far behind in areas such as health service and education. This study aimed to obtain and analyze the current genetic knowledge and attitudes that are in place amongst primary care physicians (GPs) in the Gauteng province of SA. It also aimed to obtain a clearer understanding of the opinions that GPs have towards GC. A questionnaire examining these topics was designed and was posted to 1091 GPs selected by choosing 1 in 9 of the GPs registered with the South African Medical Association in the Gauteng area. Less than 7% of this targeted group replied - a possible indication in itself of the general apathy and insecurity that doctors may feel towards the topic of genetics. Fifty face-to-face interviews utilizing the questionnaire were also conducted so that first hand responses could be recorded. The data collected from all these responses indicate that SA GPs have a poor genetic knowledge with a mean overall score of 57% in the genetic knowledge section of the questionnaire. Whilst genetics and GC is held in high regard, and deemed important in the primary care context, it is generally viewed as out of the GPs realm and in general they do not feel informed enough in this area to become involved in genetics. In this study 92% of GPs felt unable to construct a family history and draw pedigrees and 94% felt unable to discuss genetic tests and risks with their patients.

Prophylactic Surgery to Reduce Cancer Risk: Attitudes Before and After the Availability of Genetic Testing. C. Skrzynia, E.M. Rohlf, J.K. Booker, H. O'Lear, L.M. Silverman, M.L. Graham, J.P. Evans. University of North Carolina at Chapel Hill; Chapel Hill, NC.

Many women who perceived their risk of breast and ovarian cancer to be elevated underwent prophylactic mastectomy (PM) and/or oophorectomy (PO) in the years preceding the availability of genetic testing. Some of these women subsequently underwent genetic testing for BRCA1/2 mutations once it was available. Little is known about how these women react to learning their mutational status. The Cancer Genetics Clinic at UNC has evaluated over 400 families in the past 4 years regarding their cancer risk. 168 women were tested. There were 32 mutation carriers, for a mutation incidence of 17%. Three of the women who learned they were negative for a familial mutation had previously undergone PM. These three women were interviewed and their reactions to having undergone what might, in retrospect, be considered unnecessary surgery, were assessed. In the current era, genetic testing is sometimes undertaken to guide a woman's decision regarding prophylactic surgery. We were interested in how the results of genetic testing are used when making a decision about the pursuit of PO. In the 19 mutation carriers who still had their ovaries the decision to undergo PO was highly age dependent. Of 11 women who were under 36 years old and were mutation carriers, none underwent PO, while of the 8 women over 35 years of age, 6 (75%) opted for PO. The discovery that one is a BRCA mutation carrier can lead to a change of attitude about prior treatment. For example, one woman in our series had undergone bilateral lumpectomies, chemotherapy, and XRT for previously diagnosed bilateral breast cancer. Upon learning that she carried a mutation in BRCA1, she proceeded with bilateral PM and PO. The decision about prophylactic surgery is highly dependent upon age of the carrier, previous diagnoses and treatment, and may be influenced by new data regarding the efficacy of prophylactic surgery in reducing cancer risk.

Amniocentesis Utilization in a Diverse Urban Population: A Profile of Patients in the 21st Century. V.B. Penschaszadeh, D. Puñales-Morejon, G. Havens, M. Cantwell. Division of Medical Genetics, Beth Israel Medical Center, New York, NY.

Information about factors that impact on women's decision-making processes regarding prenatal diagnosis is important for genetic counseling. Various studies have examined women's experiences with amniocentesis and have described specific factors as having a role in women's testing decisions. However, most studies were limited by sampling homogenous populations, analyzing small numbers of patients or showing methodological inconsistencies in data collection. This study was conducted to assess utilization of amniocentesis in a diverse urban population and to correlate its use with demographic and medical factors. Variables included: referral reasons, indication for amniocentesis, maternal age, race, ethnicity, education, occupation, marital status, partner attendance, gestational age of current pregnancy, parity, planning of pregnancy and amniocentesis decision. Data was collected on 1227 patients referred for prenatal genetic counseling primarily for age-related risk or abnormal prenatal screening. Seventy-eight percent of the 1063 patients for whom amniocentesis was medically indicated, and 18 percent of the 164 patients for whom it was not indicated elected to have the procedure. Blacks and Latinos were found to be less likely than Whites or Asians to have amniocentesis. In addition, those who were married, were professionals, had medical insurance, came to counseling with their male partners or planned their pregnancies, were more likely to have amniocentesis performed. Furthermore, patients who had amniocentesis tended to be older, have at least a bachelor's degree and be at an earlier gestational age. A multivariate logistic regression indicated that education was the primary factor associated with having amniocentesis performed. Data from this study can be helpful for genetic counselors and geneticists to identify which variables are useful in predicting a woman's decision to use or not use prenatal diagnosis. It is anticipated that the results from this investigation will assist in better understanding the prenatal diagnostic decision-making process.

Cultural and Linguistic Considerations in Development of Genetic Educational Materials in a Predominately Mexican American Population in South Texas. *M. Aguilar¹, S. Kolb², P. Visio³, J. Livingston³, C. Aguirre³, C.I. Kaye³.* 1) CHRISTUS Santa Rosa Health Care; 2) University of the Incarnate Word; 3) University Texas Health Science Center at San Antonio.

Children in South Texas, of whom 76% are Mexican American (MA), fail to comply with recommendations for genetic services. Over 63% of families of children with Spina Bifida (SB) are not compliant with recommendations for urinary care; 65% of children with SB are obese; 53% of children with Metabolic Disorders and 72% of children with Diabetes Mellitus are in poor dietary control. A sample of 27 MA families was selected based on clinic attendance rate of 80% and poor compliance with medical recommendations. Each family was assessed for Spanish/English health literacy, values/attitudes, and preferred learning methods. The Test of Functional Health Literacy in Adults (TOFHLA) was used to assess literacy. A Family Learning Preferences (FLP) tool inquired about previous teaching received by the family, and preferred "ways of learning." Family specific culturally based health factors were assessed using the Health Beliefs Questionnaire - Revised (HBQ-R). The TOFHLA scores indicated that 31 % of the subjects interviewed had an inadequate to marginal functional health literacy level, while 69 % of subjects had an adequate functional health literacy level. The FLP indicated that "best liked way of learning" was in small groups (4-5) and by videotapes and demonstration. The "least liked way of learning" was in large groups (10 or more), by written materials, and teaching done by other parents. Using the HBQ-R Likert Scale, there were statistically significant positive correlations between acculturation and education ($r = .648$); between literacy and education ($r = .460$); and a negative correlation between folk beliefs and education ($r = -.570$). Preliminary findings suggest that attention to reading level and preferred learning styles is needed for development of culturally/linguistically appropriate genetic educational materials to insure successful outcomes. This project is supported by MCHG Project # 97-11.

Public Attitudes about the Importance of Genetic Factors in Determining Health. *A.S. Brown, S.S. Wang, M.L. Gwinn, M.J. Khoury.* Office of Genetics and Disease Prevention, National Center for Environmental Health, Centers for Disease Control, Atlanta, GA.

With the increase in the amount of genetic information readily available to the public and the integration of DNA-based diagnostics into health care, it is important to understand which characteristics determine how people respond to genetic information. To assess public attitudes about the relative importance of genetic factors in determining health, we examined data from the 1998 American Healthstyles Survey. Individuals participating in this survey of health attitudes and behavior are representative of adults in the U.S. with regard to age, gender, marital status, race, income, region, household income, and residence (urban, suburban, rural). As part of this survey, participants were asked whether they agreed with this statement: "Genes determine more of a person's health than other things, like their behavior or their environment". Of 3,130 survey participants, 2,639 (84%) completed this question. Overall, 37% agreed, 26% disagreed, and 36% neither agreed nor disagreed with the statement. Those who agreed were more likely ($p < 0.05$) to: 1) have a high school education or less (OR = 1.4), 2) have household incomes less than \$50,000 (OR = 1.3), 3) be widowed (OR = 2.9), and 4) be older than 45 years old (OR = 2.0) ($p < 0.05$). These people were also more likely than others to believe that genetic research will prevent future disease (OR = 2.0). These findings suggest that characteristics such as education, income, and age may contribute to a person's attitudes about the importance of genes in determining health. Such people could respond with either a fatalistic or overly optimistic attitude upon learning the results of a genetic test. Providers of genetic information should be aware of these characteristics as they design education programs about the use of genetic information to make informed decisions concerning genetic testing and personal health behaviors.

Genetics and Emergency Room Medicine. *P.F. Giampietro, P. Kumar, J. Radhakrishnan.* Dept Pediatrics, Lincoln Hospital and Mental Health Center, Bronx, NY.

Patients with genetic disorders pose a special challenge for Emergency Room (ER) medical staff because they have more frequent admissions, longer stay and increased morbidity and mortality and complex health needs. A retrospective study of 15,258 Pediatric Emergency Room (ER) charts at our institution was undertaken in order to determine the prevalence and patterns of presentation of previously diagnosed and suspected genetic disorders among pediatric ER visits over a period of time between October, 1998 through February 1999. Patients with asthma and diabetes were excluded from our study group since referral for genetics evaluation would not be expected to impact their overall management. 318 patients (2.08%) had known or suspected genetic diseases. Known genetic disorders were observed in 80 patients (25.15%) which were further classified into single gene disorders 69(86.25%), chromosomal disorders 3(3.75%) multifactorial disorders 6(7.5%) and others 2(2.5%). The remaining 238 patients (74.85%) had presenting complaints/diagnosis that suggested the possibility of an underlying genetic disorder requiring further evaluation. These included development and behavior issues 84 (35.2%), CNS problems 59 (24.8%), skin disorders 25 (10.5%), cardiovascular system problems 20 (8.4%), hematological problems 19 (8.0%), ENT problems 10 (4.20%), psychiatric issues 6 (2.52%), musculoskeletal problems 6 (2.52%), endocrinologic disorders 4 (1.68%), metabolic 3 (1.26%), renal 1 (0.42%) and gastrointestinal disorders 1 (0.42%). Based on prior studies reported in the literature for inpatients, we believe the prevalence of 2.08% represents an underestimate, which is most likely due to incomplete secondary diagnosis documentation. A referral to genetics was not listed in the management plan for any of these patients. Since many genetic disorders are associated with acute and chronic health care issues encountered in an ER setting, these data suggest a need for genetics professionals to target ER medical staff for education regarding the acute multi system presentation of genetic disease and the important role that genetic specialists have in the long term management of patients with genetic disorders.

Gene Therapy in Genetic Counseling: a survey of knowledge and attitudes. *P.E. Gregory, S. Balsom.* Div. Human Cancer Genetics, Ohio State Univ, Columbus, OH.

Advances in gene therapy for both inherited and acquired disorders bring DNA-based therapies closer to being incorporated into clinical care. Consequently, genetic counselors will increasingly be called upon to explain gene therapy and its applications to their patients. In 1992, we attempted to assess the attitudes and aptitudes of genetic counselors on this subject. This study is a follow-up to that survey and is designed to identify changes that may have occurred in the past eight years. The current survey was conducted via e-mail with a third-party to anonymize the responses. Using the internet was not only more cost-effective but also increased the participation rate. Surveys were e-mailed to every genetic counselor who is a member of the National Society of Genetic Counselors, the responses were anonymized and the data entered into a database for analysis. The previous study had shown that there was a direct correlation between recent matriculation and knowledge of current gene therapies. These differences held true in this second survey. Questions concerning knowledge of gene therapy techniques showed an inverse correlation between the number of years in practice and the percentage of correct answers. The more experienced counselors, with 10 -15 years of experience, were four times less likely to include any information about gene therapy in their counseling. However, 100% of this group felt that "information on human gene therapy should be incorporated into genetic counseling training curricula. This statistic has remained the same since the original survey. Interestingly, the most substantial differences were observed in counselors' attitudes toward gene therapy. Experienced counselors were more concerned than any other group about the "unknown risks of therapy" (95% vs 62% and 68%). New counselors (with less than 5 years experience) were least concerned about gene therapy on embryos. Our results suggest that many of the findings in the original survey have held true, despite many changes in gene therapy in the past 8 years. Since gene therapy is rapidly changing, there is an even greater need for continuing education programs for genetic counselors in this area.

A Prototype for Clinical Training in Medical Genetics for Third Year Medical Students. *A.E. Greb¹, G.L. Feldman^{1, 2}*. 1) Center for Molecular Medicine and Genetics; 2) Departments of Pathology and Pediatrics, Wayne State University School of Medicine, Detroit, MI.

While advances in medical genetics are causing fundamental changes in the way physicians think about health and disease in their practice of medicine, similar changes in medical school curriculums have been slower to develop. The average number of hours devoted to coursework in medical genetics is 29 (based on a 1995 survey of U.S. and Canadian medical schools performed by the Association of Professors of Human and Medical Genetics). Most medical genetics courses are taught during the preclinical years, prior to the medical students knowledge of clinical pathophysiology and patient care. At most institutions, there is no formal mechanism during the 3rd or 4th years to reinforce the genetics information introduced during their preclinical introductory course. Through funding received from the Michigan Department of Community Health, we developed an interactive computer-assisted learning exercise using a problem-based approach to integrate genetic principles into a primary care clerkship. Students are provided information about a hypothetical case, including a referral letter, family history and physical symptoms. The student works through a differential diagnosis and decides upon appropriate tests and is asked to provide the family information about prognosis, etiology, recurrence risks, available options and appropriate resources. Ethical, legal and social issues are included in the case study. Students learn to use available Internet sites such as OMIM, GeneClinics, GeneTests and the Genetic Alliance to assist in their decision-making process and patient counseling. A post-test is administered to evaluate whether the student achieved the learning objectives. We have initially incorporated this as part of the 2-month internal medicine clerkship and will present the prototype in an interactive presentation. We plan to develop similar models in other primary care clerkships, such as pediatrics, obstetrics/gynecology and family practice. This type of resource can be easily updated, offering a fluid model of interactive genetics integration into medical school education.

Information needs of women at risk for hereditary breast cancer. *W.F. Cohn¹, G. Fraser², S.M. Jones³, M.E. Ropka¹, S. Miesfeldt⁴.* 1) Dept. of Health Evaluation Sciences; 2) Dept. of Anthropology; 3) Cancer Center; 4) Dept. of Int Med, University of Virginia, Charlottesville, Va.

Problem: Breast cancer survivors facing the possibility that their cancer may be associated with an inherited syndrome may have unique information needs. **Purpose:** To identify and understand information needs related to concern for hereditary breast cancer (HBC). **Method:** A multi-method approach combining qualitative data from in-person interviews and quantitative data from mailed surveys. 314 women with a history of breast cancer, recruited from 34 Virginia Hospitals, completed a family history questionnaire to identify those at presumed risk for HBC. Of the 138 respondents at risk for HBC, 20 from diverse backgrounds were selected for an in-depth interview. Interviews were conducted using an interview guide about HBC issues developed by literature review and clinical expertise. The qualitative data were content analyzed yielding information themes. All 138 at-risk women were mailed a survey to assess knowledge, beliefs and attitudes regarding HBC. Survey topics included: 1) health history and experience; 2) knowledge about HBC; 3) beliefs about breast cancer causes; 4) information resources about HBC. **Results:** Content analysis of the interviews identified the following major themes related to breast cancer information: needs, timing/delivery, sources, comprehension. Questionnaire results revealed that only 53% of women reported looking for information regarding HBC following their diagnosis. Women who sought information were interested in: (1) how their diagnosis affected their children's risk, 41%; (2) how their diagnosis affected other family members' risks, 23%; (3) DNA testing information, 13%; (4) treatment differences for inherited breast cancer, 13%; (5) genetic counseling, 10%. Most women, 78%, reported that they were able to find the information they sought. **Conclusions:** Many at-risk breast cancer survivors may not pursue information about HBC. Among respondents there was a greater interest in information concerning their children's breast cancer risk than in information about genetic counseling or testing. Work supported by NHGRI (R29 HG01554).

The Genetics of Psychiatric Disorders: Clients Perspectives on and Expectations of Genetics. *K.K. Milner, K. Neudoerffer, L.B. Smith, A.C. Madeo, E.M. Petty.* University of Michigan Medical School, B1D102, UH, Ann Arbor, MI 48109-0020.

Genetic contributions to chronic mental illnesses (eg. schizophrenia, bipolar disorder) are complex and poorly defined. Pedigree, twin, biochemical, and genetic analyses suggest that genetic factors contribute to these conditions. Some specific alterations (eg. del 22q) are associated with an increased predisposition to psychiatric problems. Previously we found that mental health care providers in Michigan rarely referred clients to Genetics. Most were unsure if genetic services were available. Interestingly, clients and/or their family members independently seek genetic services for recurrence risk counseling, teratogen information, and sometimes, gene tests. While this is not surprising given media portrayals of psychiatric genetics, we were interested in learning more about the level of understanding and related expectations people with mental illness and their families and friends have regarding genetics. We administered a written survey in Southeast Michigan to examine their genetic literacy, understanding of the causes of psychiatric conditions, knowledge about genetic services, and desire for such services. Most respondents (n=100) noted that a variety of genetic and non-genetic factors contribute to mental illness but could not define basic genetic concepts. Many saw potential benefits of genetics for themselves or family members. Most did not know if specific genetic tests were available in psychiatry. Most wanted to obtain new information about genetics from physicians rather than media. In conclusion, even though people affected by chronic mental illness demonstrate a limited understanding of genetics they want to better understand genetic contributions to mental illness and desire genetic services. This may increase as knowledge about the human genome leads to genetically based prognostic and therapeutic options in psychiatry in the future. Thus, it will be critically important to consider the needs of the people most directly affected by this knowledge as we develop health and educational initiatives utilizing new genetic knowledge.

Genetics education for health professionals: What works, What doesn't work and Why. *E.V. Lapham¹, J.O. Weiss², C. Kozma¹, M.A. Wilson², J.L. Benkendorf³*. 1) Dept Pediatrics, Georgetown Univ - CDC, Washington, DC; 2) Genetic Alliance, Washington, DC; 3) Dept Obstetrics/Gynecology, Georgetown University, Washington, DC.

A three-pronged approach to genetics education of health professionals has been carried out for three years (1997-2000) as part of the Human Genome Education Model (HuGEM) Project of Georgetown University and the Genetic Alliance. Seven national health professional organizations collaborated with HuGEM to do genetics education for national staff and boards, educators and other leaders, and practitioners. The seven associations were American Dietetic Association, American Occupational Therapy Association, American Psychological Association, American Physical Therapy Association, Council on Social Work Education, and National Association of Social Workers. Preliminary outcomes show differing results both within and between the three approaches. Following orientation sessions with 130 national staff and board members, professional culture, commitment and outside endorsement influenced the extent to which each organization made progress toward integrating genetics into activities and policies. Follow-up activities of 60 educators who took a 5-day genetics course also varied and depended upon method of selection, organization responsibility, and technical assistance. While 1200 professionals attended the 36 training workshops, about one-third signed up to do training. However, the majority of practitioners did not follow through because their supervisors did not see the relevance to practice, colleagues were not interested, family and personal crises intervened, or they forgot. Educators attending the workshops did use the video and other materials to educate students and colleagues. From this analysis, a recommendation is made to start education with national boards and staff, provide intensive courses for educators selected by the associations, but focus training workshops on educators who have the experience and outlets to educate both students and colleagues. Educating field supervisors may be a better way of reaching practitioners.

Genetics Program for Nursing Faculty: Nursing school curriculum outcomes. *C.A. Prows, N. Johnson, C. Hetteberg, K.K. Latta.* Childrens Hospital Med Ctr, Cincinnati, OH.

The Genetics Program for Nursing Faculty (GPNF) is a multifaceted program that aims to increase faculty knowledge about genetics and to increase the amount of genetics content in entry-level nursing education program curricula. From 1997 to 1999, three on-site Genetic Summer Institutes (GSIs) provided genetics education and curriculum resources for a total of 94 nursing faculty participants representing 85 different nursing schools in 27 different states and the District of Columbia and Puerto Rico. Pre-test and post-test tools were used to measure change in knowledge. Statistically significant improvement ($p < 0.01$) in post test scores was demonstrated. Change in nursing curricula genetics content was determined using a pre-GSI and post-GSI curriculum survey. A base line curriculum survey was sent to each participant prior to the 1997 and 1998 GSIs and shortly after the 1999 GSI. A post survey was sent in April, 1999 to the 1997 and 1998 GSI participants. Pre-GSI surveys were completed and returned by 53 participants (90% return rate). Completed post-GSI surveys were returned by 39 out of the 53 participants who returned a pre-GSI survey (74% return rate). Paired sample t tests were used to measure change in the average total number of genetic topics and conditions taught within the 39 nursing schools from time 1 (pre-GSI) to time 2 (post GSI). Paired sample t tests were also used to measure change in average total time spent on genetic topics and conditions from time 1 and time 2. There was statistically significant ($p < 0.001$) improvement in the mean total number of genetic topics and conditions (18.6 at time 1 and 23.6 at time 2 with a t score of 4.4) and statistically significant ($p < 0.001$) improvement in the mean total time spent on genetics topics and conditions (13.3 hours at time 1 and 22.5 hours at time 2 with a t score of 3.7). The average amount of time spent per program on most genetic topics and genetic conditions increased. Therefore, schools were improving the scope of content covered and the amount of time devoted to genetics.

PHYSICIAN ASSISTANTS' KNOWLEDGE OF GENETICS. *C. Tarantina, T.W. Kurczynski.* Mercy Children's Hospital, Medical College of Ohio, Toledo, OH.

A questionnaire modified from one used to assess physicians' knowledge of genetics and genetic tests (K.J. Hofman et al, Acad. Med. 68:625-632, 1993) was used to survey physician assistants in regard to genetics knowledge and attitudes. The modified questionnaire was mailed to a national sample of 500 randomly selected physician assistants who were members of the American Academy of Physician Assistants (AAPA). The survey included demographic and educational information as well as questions pertaining to genetic risk assessment, pedigrees and hypothetical clinical situations. A total of 81 physician assistants (17%) responded. Twenty-six percent had a required genetics course as part of their training program. The respondents correctly answered 59% of the knowledge questions but 77% felt their training programs did not adequately prepare them for handling genetic issues. Eighty-six percent of the respondents indicated it was important to detect potential or actual genetic conditions. Although the response rate was low, the majority of respondents recognized the increasing importance of genetics in the care of patients and improving their preparation for handling genetic issues surrounding their patients.

DNA goes to school! A pioneer educational project in Rio de Janeiro, Brazil. *M. Rufier, M. Lachtermacher.* DNA goes to School!, Rio de Janeiro, Brazil.

In the beginning of this new century, headlines about DNA catch our attention everyday. As the Human Genome Project approaches completion, it is critically important to bring science closer to the society, to have educational projects that focuses on DNA and to stimulate discussions of the issues inherent to the introduction of this new technology in our society. For this reason, we have developed DNA goes to school, a pioneer educational project launched in 1999 in Rio de Janeiro, Brazil. The project was developed in order to provide an opportunity for high school students to have a closer contact with DNA science. It combines hands-on DNA experiments with a variety of other activities dealing with DNA and the "genetic era". DNA goes to School! is a mini-molecular biology unit that fits inside a small suitcase and it literally goes to different schools. During the first year of establishment, the unit went to several high schools in the Rio de Janeiro area and more than 200 students have attended to the course. The complete course is a 12 hours class divided into 4 different sections of 3 hours and is limited to a group of 12 students. In the first class the students perform DNA extraction from E.coli. By performing the step-by-step procedure, they get important insights about DNA. The second section focuses on restriction enzymes. In this section they digest the DNA extracted in the class before and perform agarose gel electrophoresis. We also have open discussions about Genetically Modified Food. The third class focuses on DNA paternity where they perform a number of activities with colorful pens that symbolize alleles. The fourth class focuses on genetic tests and DNA sequencing. They also have discussions where they learn about the power and the limits of genetic tests. We have evaluated the course and 98% of the students pointed that the course is adequate for their level of knowledge, it has clarified important concepts and it has been a unique opportunity to have discussions about different issues concerning the "new genetic" era.

Cultural and ethical issues associated with subjects' participation in genetic family studies. *N.H. Arar¹, R. Plaetke¹, H. Hazuda², R. Duggirala², P. Pergola¹, B. Kasinath¹, M. Stern², H. Abboud¹.* 1) Dept Medicine, Div Nephrology, Univ Texas Health Sci Ctr, San Antonio, TX; 2) Div Clinical Epidemiology.

There is evidence of a genetic susceptibility for the development of diabetic nephropathy (DN) because it clusters in a subset of families with type 2 diabetes mellitus (T2DM). Little is known about subjects' opinions regarding this clustering and their participation in genetic family studies (GFS). Understanding cultural and ethical issues associated with GFS will enhance the enrollment process, and help to establish strategies for genetic counseling of patients with T2DM, DN, and their relatives. We will: (1) determine whether subjects' beliefs about the hereditary aspects of DN influence participation in GFS, (2) explore subjects' awareness of ethical issues related to their participation, and (3) analyze ethnic differences in participation in GFS. This study will combine qualitative and quantitative methods. In Phase I (qualitative), semi-structured interviews will be performed with 60 participants. Interviews will be tape-recorded, transcribed and content analyzed. We will recruit subjects from the ongoing Family Investigation of Nephropathy and Diabetes (FIND) study at UTHSCSA. In Phase II (quantitative), we will develop a quantitative questionnaire that will be tested, validated, and applied on participants in our Center. Then, it will be mailed to a larger sample of patients enrolled in other centers of the FIND study. Informal interviews at our dialysis unit suggest that patients are willing to participate in the FIND study because they want to learn more about their T2DM. Patients have also recognized that T2DM clusters in their families, and perceived this clustering as an important risk factor. However, patients did not view DN as a genetic disease, and were not aware of its clustering in their families. We will present: the rationale for using a combined qualitative and quantitative methods, ethnographic reports about subjects' experience regarding participating, and examples of family pedigrees to illustrate the clustering of DN in families. The ascertainment of DN families has been funded by a grant of the San Antonio Area Foundation and the NIH (DK-57295).

There is a need for graduate training in the ethical aspects of genetics research. *S.I. Bidichandani.* Master Teachers' Fellowship Program, Baylor Col of Medicine, Houston, TX.

Recent advances in genetics have raised a multitude of ethical, legal and social issues. Students enrolled in the Molecular and Human Genetics graduate program at the Baylor College of Medicine were surveyed in order to perform a "needs analysis" for formal training in the ethical aspects of genetics research. An anonymous, web-based survey was designed and utilized for this purpose. Responses were obtained from 49 of the 56 students surveyed.

A need for graduate training in the ethical aspects of genetics research was established based on the following data: (a) 43 / 49 (88%) students strongly agreed with Statement A: "It is important for the Genetics program to teach students to recognize and address ethical issues in genetics as part of graduate student training", (b) 30 / 49 (61%) strongly disagreed with Statement B: "Teaching/discussion of ethical issues in genetics in the current curriculum is adequate", and (c) The mean response of all 49 respondents to Statements A and B, 4.34 and 2.36, differed significantly ($P = 1.6 \times 10^{-16}$), indicating a substantial discrepancy in the perception of the importance of ethics training versus the adequacy of the current curriculum in providing it. A varied student population was ascertained with respect to their individual research activities. The 49 respondents reported having at least some involvement with the following activities: (a) Gene identification (71%), (b) Use of human DNA samples as controls (55%), (c) Genetic testing (39%), (d) Clinical genetics (29%), (e) Gene therapy (24%), (f) Genome sequencing (20%), (g) Commercialization of genetic tests (16%), and (h) Genetic counseling (14%). An effective graduate course would therefore have to be broad-based. Of the various educational formats, "small group discussion" was the most preferred forum to address ethical issues; significantly preferred over "lectures" ($P = 3.7 \times 10^{-5}$), "journal club" ($P = 0.0003$) and "web-site" ($P = 8.3 \times 10^{-11}$). Our data indicate a need for training genetics graduate students in the ethical aspects of genetics research, and may be pertinent to other programs similar in scope to the one at Baylor.

Evaluation of an Educational Intervention for Pediatric Providers on Genetic Testing and Ethics. *M.J. O'Neill, L.A. Demmer.* Pediatrics, U. Mass Memorial Health Care, Worcester, MA.

OBJECTIVE: To evaluate the short and long term effectiveness of a pediatric educational session on genetic testing and related ethical issues. **BACKGROUND:** Advances in genetic technology will ultimately pose many new challenges for pediatricians. The advent of genetic testing is also raising many ethical questions. Surveys have demonstrated a strong desire on the part of primary care physicians for continuing educational efforts in this area. **METHODS:** A three hour educational session was conducted for 22 pediatricians. The focus was on the availability and appropriateness of genetic testing. Format consisted of a didactic session, role play and discussion. Pre and post session surveys containing 7 genetics/ethics questions were distributed to all participants. The same survey was sent to the participants 9 months later to evaluate long term retention of information. Nine month data was received from 8 participants. **RESULTS:** Ethics questions were graded as to whether they were answered in accordance with standards used by most geneticists. The pre and post surveys demonstrated improvement for 6 of 7 questions. The average number of correct responses for the seven questions was 47% for the pre-test, 71% for the immediate post-test and 67% for the 9 month follow-up. **CONCLUSIONS:** Pediatricians demonstrated fair knowledge of genetic technology and related ethical issues, which improved with a focused educational session. Knowledge was well retained after nine months, at least by those choosing to respond to the long term survey. Ethical principles emphasized during the session were retained more consistently than details of testing techniques. This demonstrates that a focused intervention can successfully increase short and long term knowledge regarding genetic testing and related ethical issues for practicing pediatricians. Future studies should be directed toward the integration of this material into everyday practice.

Maternal serum screening from the patient perspective - a pilot study. *M. Care*¹, *S. Conacher*², *C. Meier*³, *A. Summers*³. 1) Clinical & Metabolic Genetics, Hospital for Sick Children, University of Toronto, Toronto, ON, Canada; 2) Prenatal Diagnosis Unit, University Health Network, Toronto General Hospital, Toronto, ON; 3) Clinical Genetics Diagnostic Centre, North York General Hospital, Toronto, ON.

Maternal serum screening (MSS) is a blood test which combines the levels of alphafetoprotein, human chorionic gonadotropin and unconjugated estriol in maternal blood, with maternal age to determine the risk of a fetus having Down syndrome, spina bifida and trisomy 18. MSS has been available to all pregnant women in Ontario since July, 1993. The service includes pre-test counselling and information and access to appropriate services following a positive test. Some concerns have been raised regarding the use of MSS in mainstream practice, including the high number of false positive, and whether women are making informed decisions.

The current study was designed to assess knowledge, information acquisition, how MSS was presented, decision-making, knowledge of whether testing had been done, motivations for testing, communication of results and level of satisfaction. A total of 70 women from prenatal education classes at Toronto General Hospital participated in the study.

The results showed that in this well-educated population, knowledge of MSS was high. However, of the 65 women whose records we were able to access, 8% did not know their true MSS status. The results also showed that 30% of women were presented with MSS as a routine test, and 21% had testing because their health care provider (HCP) told them to. This project also illustrated different practice patterns between obstetricians and family practitioners. A significantly higher number of obstetricians presented MSS as a routine test ($p < 0.02$) and a greater number of women being cared for by obstetricians reported having the test because their HCP told them to ($p < 0.001$). Although these results cannot be generalized due to the small numbers and high education level of this sample, they indicate that education about the importance of informed decision-making should continue to be a priority.

Ascertainment effort in genetic research studies for common diseases. *A.E. Bazyk, E. Neumeister, B. Lynch, P. Watson-Clevenger, L. McFarland, J.L. Haines.* Program in Human Genetics, Vanderbilt Univ, Nashville, TN.

As we become more aware of how genetic factors play a role in most common diseases, increasing numbers of researchers are trying to identify the responsible genes. An under appreciated component of such gene mapping studies is the labor-intensive effort of ascertaining and collecting families. To quantify the effort involved, 4 coordinators of the Family Ascertainment Core of the Program in Human Genetics at Vanderbilt University kept logs for 2 months of the year (Nov. 1999 and Mar. 2000) to determine time and effort spent in ascertaining and collecting families. The focus of the logs was on the amount of time spent on the phone, in outpatient clinics, on field trips. The 4 coordinators spent an average of 60.6 hrs/month on the phone; 15.2 hrs/coord/month. This included calls to patients, family members, physicians, nursing homes and averaged 52.9 phone calls/coord/month. Time in outpatient clinics averaged 34 hrs/month for the 4 coordinators; 8.5 hrs/coord/month. Time on field trips, including travel and time with the family, averaged 116.2 hrs/month for the core; 29.0 hrs/coord/month. Performing medical record searches to ascertain new families, averaged 31.5 hrs/month for the core; 7.9 hrs/coord/month; searching a total of 816 records in the 2 months. The core averaged 134 letters each month to participants and physician offices; 33.5 letters/coord/month. The core sent a total of 4390 letters in 3 mass mailings for recruitment. We received a total of 590 responses, a 13.4% response rate. Each coordinator spent an average of 60.6 hours each month, about 1/3 of their time, on the phone, in clinics, on field trips, or searching medical records. Time spent writing letters, reading medical records of participants, database entry, pedigree construction, and organization of field trips, required about 2/3 of each coordinators' time. The core collected an average of 41.5 participants each month; an average of 10.4 participants/coord/month. Actual labor time spent to collect one participant averaged 5.8 hours. Ascertainment and collection for genetic linkage studies on common diseases is time consuming, averaging 23.2 hours to collect a family of 4 members.

Evaluating Consent Materials for Pharmacogenetic Research. *S.C. Ray, E.C. McPherson, T.E. Arledge, P.K. Manasco.* Division of Clinical Genetics, Glaxo Wellcome Inc., RTP, NC.

The informed consent process is one of the cornerstones in the clinical development process. There can be significant difficulty in explaining the benefit, risks and procedures of a clinical study to patients. Adding a pharmacogenetic (PG) component to a clinical study can compound these challenges. Given these challenges, GlaxoWellcome chose to develop a brochure and video to help increase patient understanding. A qualitative research study was conducted to evaluate the clarity and effectiveness of the consent form, brochure and video developed to educate patients about PG research. To evaluate the materials, 54 respondents were recruited from five metropolitan cities: 57% lived in the US and 43% lived in the UK. About half of the respondents (48%) had previously participated in a clinical study. The materials were presented to respondents in order to solicit feedback via individual interviews. Respondents were asked to highlight on the consent form any areas of confusion or concern. Respondents were divided in their overall opinion of the consent form. The main area of concern with the consent was the confidentiality of the DNA samples. Although the majority made a choice about which part of the research to participate in, some did not clearly understand their options. Next respondents were asked to highlight on the brochure any areas of confusion or concern and anything that was particularly liked. The majority said that they liked/thought the brochure good or excellent. After reading the consent form and brochure, the percentage who understood the research options increased by 19%. Lastly respondents watched a video that explained the research. They were asked to write down on a notepad any parts that were confusing or noteworthy. Overall, reactions to the video were very positive. Having read the consent form and brochure and seen the video, the percentage who understood the research options increased by 36%. To supplement the PG consent, it is recommended that patients have access to additional educational materials. Materials should be given to the patient prior to the research visit so there is sufficient time for review.

Psychosocial issues of burden for genetic conditions in the Chinese population. *T.T. Chiu¹, D.H.K. Chui², C. Shuman¹.* 1) Division of Clinical & Metabolic Genetics, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; 2) Provincial Hemoglobinopathy DNA Diagnostic Laboratory, McMaster University Medical Centre, Hamilton, Ontario, Canada.

Health services which address medical and emotional needs such as social work, psychology, and genetic counseling may be under-used by the Chinese community due to issues of privacy, protection of family, and avoidance of the stigma of illness. It was anticipated that this community would perceive a greater burden for genetic conditions, because of issues unique to their cultural framework, when compared with a control population with a similar risk for the condition. A questionnaire was developed to ascertain the perception of burden for b-thalassemia, a genetic disorder relevant to the Chinese community given the gene frequency in this population. Low scores for a set of questions pertaining to disclosure and help-seeking behaviors indicated a reluctance to seek help or to share information with others. Additional questions examined perception of burden for individuals in the hypothetical event of having a child with thalassemia, and were scored with a Likert-type scale. This survey was distributed to individuals of Chinese and Mediterranean descent to determine if there were differences between these two communities. As expected, the Chinese study sample scored consistently lower than the Mediterranean study sample for all the questions related to disclosure and help-seeking behavior, and higher on most of the questions regarding perception of burden related to having a child with thalassemia. Trends in the data indicate that the Chinese population may feel a greater sense of burden for genetic conditions such as thalassemia when compared to a control population; however the majority of differences between the two groups were not statistically significant. Factors such as the scoring system design, sociodemographics of the study population, and use of hypothetical situations in ascertaining perception of burden may have confounded the attempt to draw clear conclusions. Additional research is indicated in order to improve access to and provision of genetic counseling for the Chinese community.

Family and individual coping in Ehlers-Danlos syndrome. *L.R. Jay¹, J. Farmer², W. Afifi³*. 1) Genetic Counseling Program, Beaver College, Glenside, PA; 2) Hospital of the University of Pennsylvania, Division of Medical Genetics, Philadelphia, PA; 3) Penn State University, Speech Communication, University Park, PA.

The present study explores family relationships, support resources, and communication patterns of individuals with Ehlers-Danlos syndrome (EDS). The purpose of the study was to determine which variables are significant predictors of family and individual coping with EDS. Information provided in a genetic counseling session often has medical, psychological and social implications for multiple members in a family. EDS is a collection of disorders that affect the skin, connective tissue, joints, and blood vessels. A survey was created using questions based on Rolland (1994), on Eunpu (1997) and with the framework of a family stress model, such as Patterson (1989). The survey measured variables such as perception of illness severity, family communication, sources of emotional, spiritual, social support, and family and individual coping. Participants were recruited from the 1999 Ehlers-Danlos National Foundation Annual conference. Seventy-six individuals with EDS completed the survey. Regression analysis was used to analyze the relationship between family and individual coping, with several potential predictors assessed by the survey. Results demonstrate that family communication, individual coping, and role in the family are significant predictors of family coping. The two predictors of individual coping are spousal support and perception of illness severity. Findings will be illustrated with participant responses. This investigation has highlighted the importance of support, genetic counseling, and family communication as predictors of family and individual coping with illness. Not only can genetic counselors provide support and education about EDS to patients, but genetic counselors can also help educate patients about the importance of family communication and relationships in coping with illness. Using tools such as genograms are appropriate methods for learning about how families communicate, helping patients understand their family's influence on their perception of illness, and their coping with illness.

Psychosocial implications of familial primary pulmonary hypertension. *E.A. Lientz, E.W. Clayton.* Medicine, Vanderbilt University, Nashville, TN.

Primary Pulmonary Hypertension (PPH) is a disorder characterized by increased pulmonary vascular resistance leading to right-sided heart failure, which affects 1-2 people per million. Of these cases, six percent appear to be inherited as an incompletely penetrant autosomal disorder (FPPH). The inherited form appears to demonstrate genetic anticipation, with earlier onset of disease in succeeding generations. Many individuals in the affected families have contributed blood samples to a registry at Vanderbilt in an effort to find the responsible genes. This study explores how members of families with FPPH learn about the disease and its inheritance, their sources of emotional support, and the impact of the disease on their lives. Of the 47 individuals who have been interviewed to date, two-thirds are women. Thirteen have FPPH themselves; 22 have blood relatives with FPPH, while 11 have experienced FPPH only in their spouse or in-laws. 75% of respondents understood how FPPH was inherited. Respondents reported wide variability in their ability to discuss FPPH with their relatives and with their friends. One-third reported having been offered a genetic test; of these, 70% accepted. Of those who had not been offered testing, two-thirds said they probably or definitely would be tested if it were available. The most frequently cited reason (>50%) to be tested was to learn about their children's risk. Almost one-third of those interviewed said there was no reason not to be tested. Among the rest, the most frequently cited reasons (each <20%) for avoiding testing were fears about impact on the family, ability to handle the results, and insurance. Despite extensive education, respondents appeared to be confused about the difference between diagnostic tests and donation of blood for research. These results, as well as those of >40 interviews still to be conducted, will be presented and their implications identified, particularly as they compare with other disorders that present primarily in adulthood.

Strategies for disseminating genetic education to the medical and nonmedical communities. *M.G. Banke¹, W.S. Rubinstein^{1,2}, E. O'Rourke¹, T.B. Tanner³, J.A. Barranger¹.* 1) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Cancer Genetics Program, UPMC Health System/University of Pittsburgh Cancer Institute and Magee-Womens Hospital, Pittsburgh, PA; 3) Clinical Tools, Inc., Chapel Hill, NC.

The Genetics Education and Counseling Program (GECF) at the University of Pittsburgh is a public health initiative for community and professional education on genetics. The goal of the program is an increased awareness of the advances in genetics and their impact on public health services and policy. The program aims to narrow the gap between what is learned in the laboratory and the understanding of that knowledge by professionals and the public. The GECF offers educational workshops, referral information, brochures, a hotline (800-640-GENE), and a comprehensive website (<http://www.pitt.edu/~edugene>). The program extends the community outreach activities of the Center for the Study and Treatment of Jewish Genetic Diseases, the Gaucher Disease Diagnosis and Treatment Program, and the Cancer Genetics Program. To continue and expand the educational services, online educational tools will be used to provide broader access and an alternative or supplement to the standard types of learning environments such as lectures, classroom instruction, and written materials. Online learning environments are being developed for genetics education for physicians, medical students, and young children. An interactive website entitled "Community Genetics: A Public Health Guide" is also in progress. Satisfaction and effectiveness of these learning environments will be measured by scores of increased knowledge, attitude change, counseling skill development, and improved self-efficacy. These learning modules will discuss genetics, genetic counseling, testing and other services, and the ethical, legal, and social implications (ESLI) that surround these topics.

Working with the media: Lessons learned from delivering predictive test results on camera. *W. Meschino¹, K. Doran¹, L. Garon².* 1) Genetics, North York General Hospital, Toronto, ON, Canada; 2) Porcupine Health Unit, Timmins, ON, Canada.

With the explosion of genetic information in recent years, media reports announcing new discoveries are a daily occurrence. Increasingly, genetics professionals are being sought to simplify this complex knowledge for the public audience. As well as being interviewed themselves, they may be asked to choose patients who will provide first-hand accounts of how these discoveries have affected their lives. Geneticists and counsellors may even be asked to contact patients who are willing to have the most intimate details of their experiences in genetics recorded on film.

We report our experience with two film documentaries in which patients undergoing predictive testing for late-onset disorders were given results of genetic tests on camera. In the first case, we were asked to select a patient at risk for Huntington disease who was willing to have her genetic counselling and test results sessions captured on film. In the second case, the 19 year-old daughter of a woman with early-onset Alzheimer disease was approached by the director of a popular TV nature series, in the course of filming a story about her family. Previous to this, she had been ambivalent about learning her genetic status.

Common themes emerging from these 2 experiences include: 1) the patient's decision about whether to have genetic testing may be influenced by the media opportunity, 2) the professional's motivation for participating may not match the film-maker's vision for the project, and therefore should be thoroughly explored before agreeing to be involved, 3) some counselling sessions with the patient alone are necessary to explore issues that the patient chooses not to reveal on camera, 4) the film crew and director may become emotionally involved in the story and attempt to interfere with the medical process, 5) potential exists for a perceived "lack of choice" to postpone testing because of commitment to the film project, 6) adverse effects on family members may occur including lack of privacy, and 7) involvement in such projects entails extensive time commitments for patients and professionals alike.

Breast Cancer Survivors' Attitudes about Educating their Children Regarding Breast Cancer Risk. *S. Miesfeldt, W.F. Cohn, S.M. Jones, J. Weinstein, M. Ropka.* Univ Virginia Health System, Charlottesville, VA.

Introduction. We explored the attitudes of a group of breast cancer survivors concerning whether and how children from a breast cancer-affected family should be educated about their potential genetic risk for cancer development. **Methods.** Women with a history of breast cancer, recruited from 34 Virginia hospitals, responded to two mailed surveys. 1) The Family History Questionnaire ascertained information allowing for the separation of participants into women with presumed hereditary breast cancer (HBC) and women with presumed sporadic breast cancer (SBC). 2) A subsequent survey included questions addressing children's concerns about their breast cancer risk and participants' preferred information sources for their children about HBC. **Results.** A total of 273 women returned both surveys. Participants' average age was 47.5 years; 91% were Caucasian, 7% were African-American and 2% were Asian-American. A total of 231 (85%) participants reported having children. Of these, 82% of participants indicated concern about their children's breast cancer risk. A total of 127 (55%) reported expressions of concern from their children about their breast cancer risk, due to the mother's breast cancer history. The mean age of children's reported expression of concern was 19.4 years (range 6-38 years) for daughters and 18.6 years (5-35 years) for sons. Of 257 respondents, 156 (61%) believed the appropriate age for their children's education about HBC to be the teens (up to age 18 years). Fewer (n=59) felt this to be 18-21 years, and a minority (n=12) felt this to be > 21 years. When asked who should provide HBC information to children (more than one choice offered), of the 254 respondents, 214 selected the child's parent(s), 166 the child's PCP, 83 a gynecologist, and 48 an oncologist. Only 30 chose a genetic counselor and 7 a geneticist. There was no difference in responses when comparing HBC women vs. SBC women. **Conclusions.** These data show concern among breast cancer survivors and their children about HBC, and raise questions about the adequacy and availability of educational resources for both daughters and sons of affected mothers. Supported by NHGRI (R29 HG01554).

The need for a genetics education program for General Practitioners in Victoria, Australia. *S. Metcalfe¹, R. Hurworth², R. Robins³, J. Newstead⁴.* 1) Education Unit, Murdoch Childrens Res Inst, Parkville, Australia; 2) Centre for Program Evaluation, The University of Melbourne, Parkville, Australia; 3) Department of History & Philosophy of Science, The University of Melbourne, Parkville, Australia; 4) The Victorian Faculty of the Royal Australian College of General Practitioners, Melbourne, Australia.

The revolution in molecular technology and the explosion of genetic information resulting from the Human Genome Project has major implications on the practice of clinical medicine in countries all over the world. In Australia, clinical genetics services are being stretched and, as has been seen in the USA and the UK, it is the primary health care domain that is being sought increasingly to provide these services. In Australia there is little published information regarding knowledge and attitudes to genetics among general practitioners (GPs). This information is essential for developing and implementing appropriate continuing education strategies, and for evaluating their effectiveness. A study was recently performed to collect data about Victorian GPs' needs for genetics education, using focus groups and surveys. GPs reported that they had some recent, if sporadic, experience in managing patients with genetic conditions, with management often being patient-driven. GPs were often confused about ordering genetic tests and admitted that their knowledge of genetics was poor. They acknowledged there was a need to strengthen their knowledge in particular areas, and reported that they needed to improve their counselling skills for families with, or at risk of having, a genetic condition. GPs made quite clear requests that genetic education programs needed to be relevant to their practice, that information needed to be concise and simple, and furthermore, that the programs should be interesting and lively. GPs asked for resources to be provided in a variety of formats, both printed and electronic, and for these to supplement case-based seminar programs. The results from this study are now being used to develop appropriate education programs and resources in genetics for GPs in Victoria, Australia.

GeneTests™ usage patterns by occupational affiliation. *N.B. Hanson¹, M.L. Covington¹, W. Neufeld-Kaiser¹, J. Edwards², P. Tarczy-Hornoch², R.A. Pagon^{1,2}.* 1) GeneTests™, Children's Hospital, Seattle, WA; 2) University of Washington, Seattle, WA.

GeneTests™ (formerly Helix) was funded by the NIH in Fall 1992 as a Genetics Laboratory Directory used primarily by genetics service providers. It was originally password restricted to healthcare providers and laboratorians. Additional funding from Maternal and Child Health Bureau in Fall 1998 permitted outreach to a broader audience. New search strategies, a primer on genetic testing, a Genetics Clinic Directory, and a teaching module have been added to the Web site. A new registration system launched in September 1999 permits aggregate examination of use patterns by occupational affiliation of our registrants. Prior to 9/99, there were 9664 "original registrants". About half were medical geneticists and genetic counselors who, as a group, were responsible for 75% of the laboratory searches. Since implementation of the new registration system, there have been 7949 new registrants, including: ~30% Healthcare Providers (physicians and allied health professionals), ~30% Public, ~20% Students/Faculty and ~14% Laboratorians. Most members of the Healthcare category are physicians, and 27% of these are geneticists. Geneticists currently use the lab directory more frequently than do other specialty physicians. However, other specialties have a significant presence as well. For instance, 23% of physician registrants are neurologists who, as a group, demonstrate fairly frequent lab directory use. Genetic counselors are the largest allied healthcare professional group and continue to show the highest use of the lab directory. Public use of the lab directory is small. The Genetics Clinic Directory has been available only since January 2000, and therefore, has been used less than the lab directory. To date (6/12/00), "original registrants" have conducted 3218 searches, while new registrant use includes: Healthcare Providers 967, Public 896, Students/Faculty 440, Laboratorians 271. Of interest is that Public use of the clinic directory is nearly as high as that of Healthcare Providers. Use of the primer and teaching module is still under evaluation and will be reported in the future.

Familial aggregation of absolute pitch. *S. Baharloo*¹, *S. Service*¹, *N. Risch*³, *J. Gitschier*², *N. Freimer*¹. 1) Neurogenetics laboratory, University of California San Francisco, San Francisco, CA; 2) Howard Hughes Medical Institute, University of California San Francisco, San Francisco, CA; 3) Department of Genetics, Stanford University, Stanford, CA.

(AP) is a behavioral trait that is defined as the ability to identify the pitch of tones in the absence of a reference pitch and is considered to be an ideal phenotype for investigating gene and environment interactions in the development of complex human behaviors. Although previous studies have pointed to the possibility of a genetic predisposition for development of AP, there has been a paucity of systematic evidence for such a genetic contribution to AP. We have now attempted to quantify the familial aggregation of AP by estimating the lambda sib (ls) for a particularly distinct form of AP, termed AP-1. Earlier we reported that music training before age 6 is required for the development of AP-1 (Baharloo et. al. 1998). Therefore for the purpose of determining ls we have estimated the prevalence of AP-1 in populations consisting of individuals who have experienced early music training. We estimated the range for recurrence risk of AP-1 in the siblings of AP-1 probands (22.6% to 43.5%). The population frequency of AP-1 was determined by pitch testing of 625 students at a music camp. An AP-1 prevalence of 2.9% was estimated among the population of students with music training before age 6. Considering the two different ranges for the sib recurrence risks, we have estimated a lower bound ls value of 7.8 and an upper bound ls value of 15.1 for AP-1. The implications of our findings for genetic mapping studies of AP-1 will be discussed.

Jervell and the Lange-Nielsen Syndrome in a Mexican Endogamic Population. *F. Huesca-Hernandez¹, J.E.*

DominguezAburto-Lopez¹, L.M. Cuesta-Cordova², C. Zamora-Gutierrez³, M.R. Romero-Gonzalez⁴, S. Frias-Cortina (RIP)¹. 1) Genetica, Instituto Nacional de la Comunicacion Humana, S. Sa. Mexico City, D.F., MEXICO; 2) Hospital General de PEMEX Tampico, Mexico; 3) Instituto Nacional de Cardiologia, S. Sa., Mexico City; 4) Hospital Central de PEMEX. Mexico.

Jervell and Lange-Nielsen syndrome has an autosomic recessive inheritance pattern that consists on congenital bilateral sensorineural hearing loss and a consistent prolonged QT interval that can cause arrhythmia. Patients with this syndrome may suffer of syncopal attacks and sudden death during the early childhood and the beginning of adolescence. The molecular basis of this disorder is explained by the action of four genes that affect essential membrane proteins that act on the electric activity of the heart, mainly exchange of ions in the potassium channel. The incidence of this syndrome varies from one to six individuals in one million. One of the challenges of genetics is to detect carriers of hereditary diseases. In the Jervell and Lange-Nielsen syndrome, the presence of the QT interval has to be either within borderline high normal range, or within higher than normal range. The QT interval is a reliable genetic marker. The cardiologic and genetic follow up process in patients with this condition is mandatory. The findings and the analysis of the segregation pattern of the pedigree of 513 individuals, in 498 positions, distributed in seven generations, including 179 audiologic studies and 179 electrocardiographic studies are presented. We also present the identification of carriers of Jervell and Lange Nielsen syndrome by segregation analysis and cardiologic tests, including EKG which has been already reported in the literature. Additionally we report a new audiometric finding. Given the low incidence of this syndrome, we consider that it is very important to have an endogamic population, which in our case was studied in a multidisciplinary way. We aim to increase our knowledge and our perspective of this syndrome in population genetics and in a future to perform molecular studies.

Genetic risk factors in pediatric ischemic stroke - a retrospective study. A. Ferro¹, J. Pinto-Basto¹, S. Barreirinho², M. Santos², C. Barbot², E. Costa², A. Sousa¹, J. Barbot², J. Sequeiros¹, P. Maciel^{1,3}. 1) UnIGENe, IBMC, Porto, Portugal; 2) HECMP, Porto, Portugal; 3) ISCS-N, Porto, Portugal.

The aim of this study was to identify hereditary thrombophilic conditions related to the occurrence of stroke in children (estimated incidence: 2.5/100,000/year). A retrospective study performed at a pediatric hospital identified 22 children with ischemic stroke, confirmed by CT and/or MRI scan. Genomic DNA was extracted and mutation detection for FV Leiden (G1691A) and FII (prothrombin) G20219A variant was carried out by PCR-RFLP with MnlI and HindIII, respectively. The thermolabile variant of methylenetetrahydrofolate reductase (MTHFR C677T) was detected by SSCP and sequencing. We found three FV Leiden heterozygous carriers (14%) and two heterozygotes with the FII variant (9%). Previous studies in the normal population in Portugal (n=38) had shown carrier frequencies of 2% and 3%, respectively. Characterization of a larger control population (n=115), matched to our sample of patients by gender and region of origin, is currently underway. The large frequency of carriers of the MTHFR polymorphism observed (54% heterozygous and 9% homozygous), was identical to the frequency described for the normal population, suggesting that this variant, as opposed to the previous ones, is not associated with an increased risk for stroke during childhood. Additionally, medical and surgical conditions known to be associated to thrombosis were present in 16/22 patients and coagulation abnormalities were biochemically detected in 10/22. In conclusion, and excluding the MTHFR variant, genetic risk factors (including hyperlipidemia and hyperhomocystenemia) were identified in 14 patients (63%), with six (27%) having two different mutations/polymorphisms. Medical/surgical risk factors coexisted with coagulation abnormalities in 13 patients (68%), medical/surgical risk factors alone were present in two (9%), and in two (9%) none of the risk factors studied were present. Our study confirms that stroke in children is commonly associated with a combination of predisposing conditions, both genetic and environmental, often with the presence of multiple risk factors.

The discovery of single nucleotide polymorphisms and inferences of human demographic history. *J. Wakeley*¹, *R. Nielsen*², *K. Ardlie*³, *S.N. Liu-Cordero*³, *E.S. Lander*^{3,4}. 1) Organismic and Evolutionary Biology, Harvard University, Cambridge, MA; 2) Biometry Department, Cornell University, Ithaca, NY; 3) Whitehead Institute/MIT Center for Genome Research, Cambridge, MA; 4) Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.

A method of historical inference which accounts for ascertainment bias is developed and applied to single nucleotide polymorphism data in humans. Ascertainment bias is the deviation in allele frequencies (from what would be observed in a random sample) caused by the discovery process. The most common type of ascertainment bias is when polymorphisms are discovered in a small sample then surveyed in a larger one. Three data sets are analyzed, and these differ in their levels of ascertainment bias. We use maximum likelihood to fit a subdivided population model which includes a possible change in effective size at some time in the past. Incorrectly assuming that ascertainment bias does not exist causes errors in inference. Two effects of this are that migration rates among subpopulations are overestimated and that the human population appears to be shrinking. Correct inferences are made when ascertainment bias is modelled properly, and these do not support any extreme changes in the effective size of the human population.

Stratification for complex disorders with genetic heterogeneity comprising both X-linked and autosomal dominant forms of inheritance: Families with familial idiopathic scoliosis. *C.M. Justice¹, N.H. Miller², B. Marosy², E.W. Pugh³, A.F. Wilson¹*. 1) Genometrics Section, NHGRI/NIH, Baltimore, MD; 2) Department of Orthopaedic Surgery, Johns Hopkins University, Baltimore, MD; 3) CIDR, Johns Hopkins University, Baltimore, MD.

Idiopathic scoliosis (IS) is a structural lateral curvature of the spine present in the late juvenile or adolescent period in otherwise normal individuals. Population studies indicate that 11% of first degree relatives are similarly affected, with the prevalence in females about 4-5 times greater than that in males. Previous studies from a number of populations have suggested autosomal dominant, X-linked and/or multifactorial modes of inheritance. As part of a large ongoing study of familial scoliosis, 200 families with at least two individuals with scoliosis have been ascertained and clinically characterized. Phenotypes include degree of lateral curvature, curve type, age of onset and sex. A genome-wide screen for 1200 individuals was performed at the Center for Inherited Disease Research. It is likely that the sample includes both X-linked and autosomal dominant forms of the disorder. Therefore, the families were stratified on the ratio of the likelihood of each family given an X-linked model relative to that of an autosomal model. Families were then ranked based on this ratio. The mode of inheritance for families in the upper tail of the distribution was assumed to be X-linked, while the families in the lower tail were assumed to be autosomal dominant. Model-dependent and model independent linkage analysis was performed on subsets based on the ranked distribution from both tails of the distribution. However, ascertainment for a trait with X-linked inheritance and subsequent linkage analysis of markers on the X chromosome can be considered to be ascertainment on both the trait and marker data, which is known to cause an increase in the type I error rate. Issues involving ascertainment of an X-linked disorder and subsequent linkage analysis of X-chromosome markers are considered. A regional test for X-linkage based on a moving average is also presented.

Vertebral scalloping in Neurofibromatosis (NF1): A quantitative approach. *E. Kwok¹, B. Sawatzky², P. Birch¹, J.M. Friedman¹, S. Tredwell².* 1) Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Department of Orthopaedics, BC Children's Hospital, Vancouver, BC, Canada.

Scoliosis occurs in 10-25% of individuals with NF1. Vertebral scalloping is often found in NF1 patients, but its clinical significance remains unknown. We measured and compared vertebral scalloping in children ascertained from a pediatric tertiary referral hospital. We used lateral radiographic films of lumbar vertebrae from 13 NF1 children with scoliosis and 14 non-NF1 scoliosis patients to derive a scalloping ratio, which was estimated by taking three width measurements from each vertebra: superior, inferior, and waist. Digital images of the radiographs were measured using electronic calipers. The average of the superior and inferior measurements was divided by the waist measurement to obtain a scalloping ratio for each lumbar vertebra.

Only the highest ratio from each patient was used in data analysis. Scalloping ratios from the non-NF1 patients were normally distributed, with a mean of 1.13 and a standard deviation of 0.03. A Z-test gave a probability of 0.003 that a randomly chosen non-NF1 patient will have a ratio greater than 1.20. Scalloping ratios from the NF1 patients are bimodal, with 31% exhibiting ratios greater than 1.20.

We conclude that this method provides a useful and cost effective way to characterize vertebral scalloping in children with NF1. There appear to be two groups of NF1 patients with scoliosis: those with vertebral scalloping and those without. Further studies are needed to define the natural history and pathogenesis of vertebral scalloping and to determine whether it contributes to the severity or progression of scoliosis in NF1 patients.

This study was funded in part by the British Columbia Neurofibromatosis Foundation. Details can be found at: <http://www.medgen.ubc.ca/friedmanlab/index.html>.

Genetic Association Study under Epidemiologic Design for the Three-Locus Genotypes --- the Susceptibility of Parkinson's Disease in Taiwan with Dopamine-Metabolizing Enzyme Genotypes of MAO-B, COMT & CYP2E1.

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The genetic study of multiple loci for disease susceptibility under a case-control epidemiology design recommends the continuous genotype frequencies in the logistic analysis. Determining a candidate genotype of disease concludes in a risk factor, a protective factor, or neither of the two. If a dummy variable of a genotype has to be employed for the genotypes of studied loci, the code of 1 or 0 for a genotype subject or not should replace a relative code system with a designated baseline system for a logistic regression. There exists no genotype for a patient or a healthy subject to be set as a baseline of the disease. The hospital based susceptibility survey in Taiwan of Parkinson's disease (PD) investigates genotype polymorphism of dopamine-metabolizing enzymes of COMT/L (Val158/Met) with gene encoded on the 22nd chromosome, MAO-B intron 13 encoded on the X chromosome and CYP2E1 encoded on the 15th chromosome. The risk factor for male PD patients is the genotype MCC311 with the mutant type for MAO-B locus, the wild type for COMT locus and the wild type for CYP2E1 locus. Neither environmental nor genetic factors are further required. For female PD susceptibility, there exists neither risk nor protective factor among genotypes of 3-, 2- and 1-locus, other than the genotype MCC2*2 of the heterozygote for both of MAO-B locus and CYP2E1 locus, a protective factor. For female PD patients, risk and/or protective factors other than these 3 loci exist and have to be identified. The survey recruited 222 PD patients and 197 individuals with age-, gender- and birth place-matched subjects. The laboratory typing for the genotype polymorphisms of MAO-B, COMT and CYP2E1 was performed using PCR-based RFLP analyses.

Methylenetetrahydrofolate reductase (MTHFR) A1298C polymorphism as a NTD risk factor for the Italian population. *V. Capra¹, P. De Marco¹, L. Arata¹, A. Moroni¹, E. Merello¹, M.G. Calevo², A. Cama¹.* 1) Lab Servizio Di Neurochirurgia, Ist. Scientifico G. Gaslini, Genova, Italy; 2) Servizio di Epidemiologia e Biostatistica, Ist. Scientifico G. Gaslini, Genova, Italy.

Periconceptional folic acid intake prevents about 70% of all Neural Tube Defects (NTDs). It has been demonstrated that alterations in folate and homocysteine metabolism may play a role, since NTD patients as well as mothers have moderately increased levels of plasma homocysteine. Homozygosity for the C677T mutation of the methylene tetrahydrofolate reductase (MTHFR) gene is associated with an increased risk for NTD, even in Italian population, which has a relatively low prevalence of NTD. However, this genetic risk factor could not account for all folic acid-preventable NTD. Another mutation in the same gene, the A1298C mutation, results in decreased MTHFR activity, which is more pronounced in the homozygous than heterozygous state. We studied the A1298C mutation in the Italian population to evaluate its role as risk factor for NTD. We used 141 children with mostly sporadic myelomeningocele, 91 mothers and 53 fathers and 111 volunteers as control group. Genetic analysis revealed homozygosity for the A1298C mutation in 15.3% of the controls versus 23.4% of the affected children, 34.1% of the mothers, 17% of the fathers. The frequency of the mutated C allele was 36% in controls versus 48% in patients, 57% of the mothers and 42% of the fathers. Thus, we observed increased frequency of the mutated allele in the NTD patients and their parents when compared with controls. The determined odd ratios showed an increased risk of 1.85 (95% CI:1.01-3.38) for the prevalence of the A/C genotype in the affected children versus controls. The odd ratio increased to 2.39 (95%CI:1.09-5.25) if the affected children have the 1298CC genotype. These data, although preliminary, indicate that both heterozygosity and homozygosity for the A1298C mutation in the MTHFR gene is associated to an increased risk in Italian population. Large groups of patients and controls need to be studied to confirm our tentative conclusion. Supported by Ricerca Finalizzata Ministero della Sanita`-Italy (grant ICS 34.1/RF 95.219).

Relationship between Wolfram Syndrome carrier status and suicide. *J. Crawford*¹, *R.D. Goldney*², *G.R.*

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Suicide is an important health problem in Australia with over 2,700 people committing suicide each year. Over 90% of those who commit suicide are suffering from some form of mental illness at the time. Our aim is to elucidate genetic factors that may play a role in suicide. Family studies in Wolfram Syndrome (WFS1) have led to the identification of the disease causing gene, wolframin. WFS1, defined by the occurrence of juvenile-onset diabetes mellitus and progressive optic atrophy, is a neurodegenerative autosomal recessive disorder with an incidence in the US of 1/100,000. Many WFS1 patients develop paranoid delusions, progressive dementia, severe depression and attempt suicide. It has also been reported that carriers of the disease have an increased incidence of clinical depression and suicide, with family studies showing a 26-fold increase in psychiatric morbidity in obligate heterozygotes compared to non-carriers. Characterisation of specific genetic changes that predispose to psychiatric illness will lead to a means of identifying at risk individuals, more precise psychiatric diagnosis and more effective treatment. It is therefore important to determine if carrier status for WFS1 is a significant factor in individuals who have committed suicide. We have a series of 100 suicides and are testing for an increased incidence of WFS1 mutations in this group as compared to blood donors who are not known to have psychiatric illness. Preliminary screening of the WFS1 ORF has been completed in approximately a third of our sample panel, and 18 sequence variants have been detected. Eight of these have not previously been reported and two cause unique amino acid changes. These results indicate the WFS1 gene is highly polymorphic and further analysis is necessary to identify disease causing mutations. We are continuing our screen with the aim of establishing a positive correlation between completed suicide and carrier status for the Wolfram Syndrome gene.

DAT AND Adult ADHD: Combining Data from Care-Controls and Nuclear Families. *P. Muglia¹, U. Jain², M. Neves-Pereira¹, J. Byam¹, J.L. Kennedy¹*. 1) Neurogenetics Section, CAMH, Clarke Site, Univ Toronto, Toronto, ON, Canada; 2) Adult and Adolescence Clinic, CAMH, Clarke Site, Univ Toronto, Toronto, ON, Canada.

Attention deficit hyperactivity disorder (ADHD) is a frequent psychiatric disorder with a lifetime prevalence of 3-5 percent in school age children. Several follow up studies have shown that roughly one third of subjects diagnosed during childhood continue to suffer from ADHD during adulthood. Convincing data support a genetic role in the aetiology of ADHD. A dopamine dysfunction in the prefrontal and striatal regions has been described in ADHD subjects. The dopamine transporter, that is responsible for the reuptake of dopamine from the synaptic cleft, is the primary site of action of methylphenidate, the treatment of choice for ADHD patients. Previous studies have investigated the dopamine transporter gene (DAT) VNTR in children with ADHD reporting an association of the disorder with the 480-bp allele. In this study we described the preliminary result from the genotyping of the DAT VNTR in two independent samples: one comprised of cases and ethnically, sex and age matched controls, and the second made up of nuclear families. The Transmission Disequilibrium Test (TDT) analysis of the genotypes in the nuclear families showed no evidence of association for the DAT VNTR with ADHD ($N = 47$; chi-square = 0.07; $df = 1$ $p = 0.81$). Similar evidence for no association was found in 30 case-controls where the frequency of the 440 and 480 bp repeat alleles were 0.3 and 0.7 in the cases respectively and 0.23 and 0.77 in the controls (chi-square of 0.38; $df = 1$; $p = 0.53$). To test the overall effect of the two independent samples, we made use of the inverse normal method as previously applied in this sample for the dopamine D4 receptor gene (Muglia et al. 2000) that gave a $z = 0.75$; $p = 0.45$. The results from our study did not confirm the associations previously shown in child ADHD and suggest that the DAT is not playing a major role in the etiology of ADHD in our sample.

Familial aggregation of neurofibromatosis 1 (NF1) clinical features. *J. Szudek¹, H. Joe², J.M. Friedman¹.* 1) Departments of Medical Genetics and; 2) Statistics, University of British Columbia, Vancouver, BC, Canada.

The relationship of genetic factors at the *NF1* locus or other loci to development of specific disease features is poorly understood. This study examines familial aggregation of NF1 features among different classes of affected relatives.

The National NF Foundation International Database contains data on 320 families with ³² members affected with NF1, including 223 sib-sib, 290 parent-child and 70 second degree relative pairs. For this study, we selected 10 NF1 clinical features: café-au-lait spots, intertriginous freckling, cutaneous, subcutaneous and plexiform neurofibromas, Lisch nodules, seizures, scoliosis, macrocephaly and short stature. The probit of each feature was set as the response in a different model. Two separate regressions were simultaneously applied with each feature as the response. One accounted for related features and covariates such as age and gender. The other measured aggregation of the response variable between affected sibs, parent-child pairs and second degree relatives.

All of the features except seizures and scoliosis appear to be familial. Among the familial features, correlations between sibs ranged from 0.30 (95% CI 0.07-0.54) for plexiform neurofibromas to 0.72 (95% CI 0.52-0.92) for Lisch nodules. Correlations between parents and children ranged from 0.24 (95% CI 0.04-0.44) for subcutaneous neurofibromas to 0.73 (95% CI 0.48-0.98) for Lisch nodules. Correlations between second degree relatives ranged from -0.18 (95% CI -0.40-0.04) for Lisch nodules to 0.59 (95% CI 0.06-1.00) for macrocephaly. Three distinct patterns were observed among the correlations for familial features: 1) Some features had similar correlations for all relationships; 2) Others had higher correlations between first degree relatives than between second degree relatives; 3) Others had higher correlations between sibs than between parents and children. These familial patterns suggest that 1) the mutant NF1 allele, 2) unlinked modifying genes and 3) the normal NF1 allele may all be involved in the development of particular clinical features of NF1, but that their relative contributions vary for different features.

Familial aggregation of coronary artery calcification in families with Type 2 diabetes. *D.W. Bowden^{1,2}, L.E. Wagenknecht³, J.J. Carr⁴, C.D. Langefeld³, B.I. Freedman², S.S. Rich³*. 1) Depts. of Biochemistry; 2) Internal Medicine; 3) Public Health Sciences; 4) Radiological Sciences, Wake Forest Univ. School of Medicine, Winston-Salem, NC.

Type 2 diabetes is recognized as a major risk factor for atherosclerotic cardiovascular disease including subclinical atherosclerosis as measured by non-invasive procedures. The role which genetic factors contribute to various measures of subclinical atherosclerosis is largely unknown. We hypothesize that subclinical atherosclerosis, measured as coronary artery calcification (CAC), will be extensive in persons with type 2 diabetes, and that its presence depends on both genetic and environmental factors. The genetic factors will result in the familial aggregation of CAC. To determine the extent of familial aggregation of CAC in the presence of type 2 diabetes, we studied 72 persons with diabetes (mean age 60 years) and 12 persons without in 33 families. CAC was measured by fast gated helical computed tomography. Other measured factors included blood pressure, body size, lipids, glycated hemoglobin, and self-reported medical history. CAC was detectable in 75% of persons with diabetes (median score = 77, range 0 - 5776). Over 20% of diabetes patients had CAC values over 400. Equally striking were the number of patients, approximately 25%, with CAC of 0 even though this group has multiple CVD risk factors. Presence of CAC was positively associated with age and increased albumin/creatinine ratio. Extent of CAC, adjusted for age, was associated with male gender, high fasting glucose, low total cholesterol and LDL, high HDL, cigarette pack-years, and history of stroke. CAC, adjusted for age and sex, was highly heritable ($h^2 = 0.53$, 95% CI: 0.028 - 1.00). In multivariate analysis, $h^2 = 0.83$ (95% CI: 0.26, 1.00), after adjusting for diabetes status, race, LDL, BMI, hypertension, and smoking. These results suggest that strong (independent) genetic factors as well as environmental factors contribute to the variance of CAC in persons with type 2 diabetes. In these data, CAC is highly heritable, an important element in designing studies to map genes contributing to both atherosclerosis and type 2 diabetes.

Coronary artery calcification is associated with two polymorphisms in the beta-2 adrenergic receptor gene. *L.A.*

*Lange*¹, *S.L.R. Kardia*¹, *L.F. Bielak*¹, *S.T. Turner*², *E. Boerwinkle*⁴, *P.F. Sheedy*³, *P.A. Peyser*¹. 1) Department of Epidemiology, University of Michigan, Ann Arbor, MI; 2) Division of Hypertension and Department of Internal Medicine, Mayo Clinic, Rochester, MN; 3) Department of Diagnostic Radiology, Mayo Clinic, Rochester, MN; 4) Institute of Molecular Medicine and Human Genetics Center, University of Texas, Houston, TX.

Coronary artery calcification (CAC), which quantifies coronary atherosclerosis, is a marker of coronary artery disease (CAD). The beta-2 adrenergic receptor gene (ADRB2), on 5q, is a candidate gene for CAC because of the receptor's role in blood pressure regulation and lipid metabolism, risk factors for CAD and CAC. We examined the relationship between quantity of CAC and the Arg16Gly and Gln27Glu polymorphisms of ADRB2 in 97 unrelated Caucasian men and 117 unrelated Caucasian women. Quantity of CAC was measured non-invasively by electron beam computed tomography in the four major epicardial arteries. The association between CAC and genotype was investigated with Tobit regression models three ways: 1) main genotype effects without adjusting for CAD risk factors (age, lipids, blood pressure, body size, glucose and smoking); 2) main genotype effects after adjusting for individual CAD risk factors; and 3) interaction effects between genotype and individual CAD risk factors. Tests were considered significant if $p < 0.05$. In men, there were no significant associations with Arg16Gly. The unadjusted main effects of Gln27Glu were significant, but were no longer significant after adjusting for most CAD risk factors. In women, there were no significant main effects of Arg16Gly, while some interactions were significant. The main effects of Gln27Glu were significant in women both before and after adjusting for most CAD risk factors. Some interactions were also significant. The Arg16Gly and Gln27Glu polymorphisms were in strong linkage disequilibrium, with a marked under-representation of Gly16-Glu27 and Arg16-Gln27 haplotypes based on estimated allele frequencies. This study suggests that ADRB2 may predict quantity of CAC in women after adjusting for established CAD risk factors.

Concordance for cardiovascular risk factors in affected sib pairs (ASPs) with early-onset coronary artery disease (CAD). *E.R. Hauser^{1,3}, V. Mooser³, S. Schmidt^{1,3}, D.C. Crossman³, J.L. Haines³, C. Jones³, B. Winkelmann³, C. Foster^{2,3}, C. Haynes^{1,3}, Z.E. Dowdy^{1,3}, S.E. Estabrooks^{1,3}, L.T. Middleton^{2,3}, P. Manasco^{2,3}, A.D. Roses^{2,3}, C.B. Granger^{1,3}, W.E. Kraus^{1,3}, M.A. Pericak-Vance^{1,3}.* 1) Duke Univ. Medical Center, Durham, NC; 2) Glaxo Wellcome, Inc., RTP, NC; 3) for the GENECARD Genetics Network.

The genetic analysis of CAD is complicated by the presence of environmental risk factors and gene-environment interactions. Population-based data suggest that the risk to relatives of an affected individual is greater at younger age of onset and that the relative contribution of environmental factors to disease susceptibility in younger individuals is lower. As a prelude to the analysis of a genome screen for CAD susceptibility genes, we examined the presence of cardiovascular risk factors in a large sample of sib pairs affected with early-onset CAD. Both sibs were required to have onset of CAD by age 50 for males and age 55 for females. ASPs were ascertained by 4 European and 2 US sites. Clinical, family and risk factor data were obtained on 456 independent ASPs from 391 families. We examined concordance for CAD risk factors, including concomitant disease, overall, by age of onset, and by site. The frequency of risk factors for the probands (and for presence in both sibs) was 79% (67%) for dyslipoproteinemia, 59% (38%) for obesity, 60% (35%) for hypertension and 19% (8%) for diabetes. The frequency of presence in the sibling given presence in the proband is 85% for dyslipoproteinemia, 65% for obesity, 58% for hypertension, and 42% for diabetes. The concordance among sibs, especially for diabetes, might be expected given the significant genetic components demonstrated for these diseases. The ASP concordance for these risk factors does not appear to be modulated by age of onset. There is also substantial ASP concordance for smoking history and weekly exercise. We will discuss the implications of these results for the analysis plan including the value of stratified or covariate-adjusted analysis. These data establish the importance of metabolic abnormalities in premature, familial CAD and the need to consider these factors in the analysis.

G Protein beta three subunit gene polymorphism and pregnancy induced hypertension. *A. kumar¹, X. Tang¹, C. Isler², J.N. Martin².* 1) Pathology, New York Medical College, Valhalla, NY; 2) Department of Obstetrics and Gynecology, University of Mississippi Medical Center, Jackson, MS.

Pregnancy induced hypertension (PIH) is a major cause of maternal and infant mortality. Earlier studies have suggested an association of the angiotensinogen gene with this disease. However, this association was not confirmed in later studies. We have analyzed the genomic DNA from 30 women with PIH and 30 pregnant women with normal blood pressure for C/T polymorphism at nucleoside 825 in exon 10 of the beta-3 subunit of pertussis toxin sensitive G-type protein. The DNA was amplified by PCR using suitable primers, cut with BsaJ1 restriction enzyme and analyzed by 3 percent agarose gel electrophoresis. Our results indicate that women with PIH had increased frequency of TT homozygotes (61 percent) as compared to pregnant women with normal blood pressure (44 percent). Our results suggest that women with TT allele have increased incidence of PIH.

Genetic screening of the lipoprotein lipase gene for mutations associated with high triglyceride/low HDL-cholesterol levels. *H. Razzaghi¹, C.E. Aston¹, R.F. Hamman², M.I. Kamboh¹*. 1) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Department of Preventive Medicine and Biometrics, University of Colorado, Denver, CO.

The lipoprotein lipase (LPL) enzyme plays a major role in lipid metabolism, primarily by regulating the catabolism of triglyceride (TG)-rich lipoprotein particles. The gene for LPL is an important candidate for affecting the risk of atherosclerosis in the general population. Previously, we have shown that the HindIII polymorphism in intron 8 of the LPL gene is associated with plasma TG and HDL-cholesterol variation in Hispanics and non-Hispanic whites (NHWs). However, since this polymorphism is located in an intron, the hypothesis is that this may be in linkage disequilibrium with a functional mutation in the coding region or intron-exon junctions of the LPL gene. The aim of this study was to initially screen the LPL coding region and the intron-exon junctions by SSCP for mutation detection in a group of 86 individuals expressing the phenotype of high TG/low HDL followed by association studies in a population-based sample of 1014 Hispanics and NHWs. Four sequence variations were identified by SSCP and DNA sequencing in the coding region of the gene, including two missense mutations (D9N in exon 2 and N291S in exon 6), one same-sense mutation (V108V in exon 3), and one nonsense mutation (S447X in exon 9). Multiple regression analyses, including these four mutations and the HindIII polymorphic site, indicate that the association of the HindIII site with plasma TG ($P=0.001$ in NHWs and $P=0.002$ in Hispanics) and HDL-cholesterol ($P=0.007$ in NHWs and $P=0.127$ in Hispanics) is independent of all other LPL variable sites examined. These observations reinforce the concept that the intronic 8 HindIII site is functional by itself and provide a strong rationale for future comprehensive functional studies to delineate its biological significance.

Importance of polymorphic variants of the TNF β gene in coronary artery disease (CAD). *U. Schagdarsurengin¹, S. Schulz^{1,2}, P. Greiser¹, E. Archoukieh¹, D. Rehfeld^{1,2}, T. Suess^{1,2}, K. Werdan², I. Hansmann¹, C. Glaeser¹.* 1) Inst. of Human Genetics, Univ. Halle, Halle, Germany; 2) Dep. of Internal Med., Univ. Halle, Halle, Germany.

TNF β is a potent cytokine, mediates proinflammatory and antiviral responses, alters the proliferative status of different cell types. It is an essential factor of the degenerative processes in the vessel wall leading to CAD. Methods: We investigated 5 polymorphic sites of TNF β (A249G, G365C and TNF β -microsatellite of intron1, C492T of exon2, C720A of exon3) in 191 patients (0,79 male, mean 51.5y) with angiographically proven severe CAD (MI: n=66, ACVB or PTCA: n=125) and 229 healthy controls (0,668 male, mean 43y) of Caucasian origin. The patient group was analyzed regarding: age at 1.MI (<or >45y), survival time after 1.MI at the moment of inclusion in the study (<or>1y), number of affected vessels, age of onset (<or>45y) and the therapy (PTCA or ACVB). Results: A249G: we found significantly more homozygous mutant allele carriers, considered to be a cardiovascular genetic risk constellation, in ACVB patients compared with healthy blood donors (0.186 vs. 0.965, $p<0.04$). MI patients who had survived the 1.MI more than one year had also more frequently the GG type compared to healthy blood donors (0.203 vs. 0.951, $p<0.04$) suggesting a possible protective influence of the mutation on adaptation after MI. C720A: homozygous mutation carriers were significantly more frequent in patients who had survived the 1.MI more than one year compared to control group (0.210 vs. 1.000, $p<0.05$). TNF β and C492T: in both cases the homozygous mutant type was significantly increased in ACVB-patients (multi-vessel disease) compared to PTCA-patients (one or two vessel disease) (TNF β : 0.036 vs. 0.904, $p<0.03$; C492T: 0.037 vs. 0.944, $p<0.03$). G365C: comparing the control group with MI patients who had survived the 1.MI more than one year we found a significantly increased frequency of C-allele carriers in the control group (0.312 vs. 1.000, $p<0.05$). Conclusion: Our results suggest the TNF β -polymorphisms as specific potential risk marker for CAD dependent on progression of the disease.

Cardiovascular disease candidate gene polymorphisms : A comparative study of frequencies between a French and an Italian population. *C. Pallaud*¹, *C. Stranieri*², *C. Sass*¹, *M. Grow*³, *G. Siest*¹, *S. Cheng*³, *P.F. Pignatti*², *S. Visvikis*¹. 1) Centre de Medecine Prventive , Nancy, France; 2) Dpt. Mother-Child-Biology-Genetics, Univ. Verona, Italy; 3) Dpt. Hum. Genet., Roche Molecular Systems, Alameda, CA.

A multilocus assay was used to genotype up to 29 variable sites in 15 genes in a French and in an Italian presumed healthy population (n=1480, n=162, respectively). These genes are involved in lipid metabolism (apoE, apoB, apoCIII, CETP, LPL, PON), homocysteine metabolism (CBS, MTHFR), blood viscosity (Fibrinogen, Factor V), platelet aggregation (GPIIIa), leukocyte adhesion (E-Selectin), and renin-angiotensin system (AT1R, ACE, AGT). Allele frequencies for all the markers were estimated in each population and compared between the two populations. The genotype distributions for each marker were in Hardy-Weinberg equilibrium in the two countries with the exception of CBS Ile278Thr polymorphism in the French population sample. Five allele frequencies were different between the two European countries: ApoB 71Ile allele (p£ 0.001), E-selectin 98T allele (p£ 0.001), E-selectin 128Arg allele (p£ 0.01) Apo E e4 allele (p£ 0.01), MTHFR 677T allele (p£ 0.01), suggesting the existence of a North-South gradient in European allele frequencies. The other frequencies were similar between the two populations, and to those observed in other European countries.

Statistical genetic analysis of a G-protein beta 3 subunit C825T allelic variant in the Old-Order Amish. G.J.

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Independent studies have suggested an association between the G-protein beta 3 subunit (GNB3) 825T allelic variant and hypertension in Caucasian individuals of Germanic ancestry with a family history of hypertension. This allele has also been associated with obesity in males of German, African, and Chinese descent. As part of an ongoing study of obesity in the Old-Order Amish, the 825T variant was determined for 157 individuals in seven families. Given that the Old-Order Amish are of Germanic descent, are genetically isolated, and have a uniform socio-economic status, this was an ideal population in which to study the C825T G-protein variant on measures of obesity and blood pressure. Although there were a substantial number of individuals with obesity in these families, the prevalence of hypertension was quite low. Standard statistical methods were used to investigate allelic and genotypic associations between this G-protein variant and quantitative measures of systolic and diastolic blood pressures and body mass index (BMI). Intrafamilial tests of association, allowing for the familial dependencies inherent in family data, were performed. The frequency of the 825T allele, allowing for familial dependencies, was estimated to be about 0.29. The data suggested significant allelic ($p=0.012$) and genotypic ($p=0.01$) associations between the 825T allele and increased systolic blood pressure in this genetic isolate.

Familiality of Subclinical Atherosclerosis in Mexican American Families. *A.H. Xiang¹, T.A. Buchanan¹, L.J. Raffen², L. Cheng², M. Quinones³, C.R. Liu¹, C.H. Liu¹, J. Diaz¹, E. Toscano¹, S. Tan¹, W.A. Hsueh³, J.I. Rotter², S.P. Azen¹, H.N. Hodis¹.* 1) University of Southern California; 2) Cedars-Sinai Medical Center; 3) University of California Los Angeles, Los Angeles, CA.

Because clinical atherosclerosis is the late stage of a complicated process, epidemiologic and genetic studies would benefit from an earlier measure of the atherosclerosis process, such as carotid artery intima-media thickness (IMT) measured by ultrasonography. Although IMT is commonly used to quantify subclinical atherosclerosis, heritability (h^2) of IMT has been less well documented. As part of an ongoing genetic study of Hispanic families ascertained via a proband with hypertension, we estimated the h^2 of IMT.

The study cohort consisted of 365 subjects from 109 families with complete measurements of IMT, age, gender, body mass index (BMI), systolic and diastolic blood pressure (SBP and DBP), and fasting lipids and glucose. The h^2 of IMT was estimated using a variance component approach; cardiovascular risk factors were included as covariates. Because the relationship of IMT and the covariates was different in parents and their offspring, statistical analyses were conducted for parent and offspring combined, and for offspring only.

The results showed that for offspring only, age and gender explained only 13.4% of the total variation of IMT; the adjusted h^2 estimate was 0.60 ($p = 0.0001$). Significant covariates SBP, TC, HDL-C and glucose explained an additional 9.4% of the variation of IMT (total = 22.8%) and increase the adjusted h^2 estimate to 0.64 ($p = 0.0001$). For parents and offspring combined, age and gender explained a larger proportion of the total variation of IMT (42.1%); the adjusted h^2 estimate was 0.38 ($p = 0.004$). Significant covariates SBP, TG and glucose explained an additional 7.8% of the variation of IMT (total = 49.9%) and reduced the adjusted h^2 estimate to 0.30 ($p = 0.005$).

The results indicate that common carotid artery IMT is heritable, supporting its use as quantitative trait for linkage studies to identify genetic determinants for cardiovascular diseases.

Vitiligo: Identification of common autosomal dominant families in large-scale population surveys. *P.R. Fain¹, D.C. Bennett², D.J. Curtis¹, C.A. Uhlhorn¹, G.L. Stetler¹, K.J. Hedman¹, A.J. Thody³, R.A. Spritz¹*. 1) Univ Colorado Health Sci Ctr, Denver, CO; 2) St. George's Hosp. Med. School, London, UK; 3) Univ. Bradford, UK.

Vitiligo is a common, non-contagious, acquired disorder, characterized by progressive patchy loss of pigmentation of skin and overlying hair. Vitiligo occurs with a prevalence of ~0.1-3 percent in most populations, and the occurrence of family clustering has been noted previously. Vitiligo is thought to have an autoimmune origin, and is associated with various other autoimmune disorders. We have surveyed two large patient groups, the Vitiligo Society (UK) and National Vitiligo Foundation (USA), and obtained data for 2040 probands and 11853 first-degree relatives, by far the largest study of vitiligo ever done. In both groups, ~85 percent of probands were non-Hispanic whites, with similar prevalence in males and females, mean age of onset ~23 yrs., and high correlation between disease duration and severity. Among first-degree relatives, the prevalence of vitiligo was ~7 percent. Most striking, in all ethnic groups 5-10 percent of probands came from families with typical vitiligo, but with early age of onset, greater severity, and apparent autosomal dominant inheritance with high penetrance. The largest such family included 14 affecteds in 3 generations with full penetrance. The prevalence of non-vitiligo autoimmune disorders was greater among probands from the USA (43 percent) versus the UK (21 percent), about half being thyroid disease. This could reflect either different rates of disease or of diagnosis in these two populations. There was no difference in the frequency of other autoimmune disorders in probands from vertical-appearing families versus sporadic cases of vitiligo. Thus, a small but significant fraction of patients with vitiligo derive from families with apparent autosomal dominant inheritance, a paradigm similar to that of breast cancer and many other complex traits. We are currently attempting to map and identify dominant vitiligo susceptibility loci, and to investigate the role of these loci in typical non-familial vitiligo as well as in other autoimmune disorders.

Frequency of depression among siblings of individuals with Autistic Disorder (AD): Effect of Race and Family History. *R.K. Abramson¹, K.A. Decena², R. Walker², H.H. Wright², S.A. Ravan¹, K.M. Wieduwilt², M.L. Cuccaro¹.* 1) Wm S Hall Psychiatric Inst, Columbia, SC; 2) Univ of South Carolina Sch of Med, Columbia, SC.

Studies have shown an increased incidence of affective disorders predating the birth of a child with AD in first and second degree relatives (Piven 1999, Bolton 1998, Smalley 1995, Abramson 1992, Piven 1991, Dumas 1991, DeLong 1988). This study examined the effects of race (Caucasian, C; African American, AA), age, gender, and family history of psychiatric illness on sibling self-report of depression. Subjects were recruited from an AD linkage study. Sibling self-report and parent report of depression in nonAD children were evaluated using the Childhood Depression Inventory (CDI) and CDIP (parent version). There were 93 surveys returned for 43 siblings (C=30, AA=13), including 30 CDIs, 35 CDIPs from mothers and 28 CDIPs from fathers. Data analysis included Pearson correlation, chi-square, and paired t-tests. No significant age or gender effects were detected. Three findings were significant: frequency of depression, race, and family history of psychiatric illness. First, depression in this group of siblings (17.2% total siblings [5/29] and 10% of preadolescent siblings [3/29]) was high compared to preadolescents (1.8%) in the general population (Anderson 1993). Second, the AA sibling and parent mean CDI scores were higher than the C sibling and parent CDI scores. There were significant differences between AA and C siblings and AA and C parents in CDI subdomain scores, indicating qualitative as well as quantitative differences in depression symptoms. Finally, CDI/CDIP scores were significantly higher where family history was positive for affective disorder/other psychiatric illness. The potential relationship between familial affective disorders and AD in the subgroup of families with affective disorders and AD has not been evaluated. Family history of affective disorder and race as stratification variables for linkage studies need to be explored.

The broader autism phenotype: Defining genetically informative dimensions. *L. Zwaigenbaum, P. Szatmari, J. Goldberg, S. Bryson, W. Mahoney, G. Bartolucci.* Pediatrics, McMaster University, Hamilton, Ontario, Canada.

Background: The Broader Autism Phenotype (BAP) refers to PDD-like traits that are found more commonly in relatives of autistic probands than controls. The standard Family History Interview (FHI) (1991; developed by Rutter and Folstein) defines the BAP along the theoretical lines of the autistic triad: communication deficits, social deficits and repetitive behaviours. However, this approach to measuring the BAP may not be the most genetically informative.

Objective: to empirically identify aspects of the BAP that best distinguish biologic (BR) from non-biologic (step- or adoptive) relatives (NBR) of probands with autism/PDD. Methods: 2nd and 3rd degree relatives of 127 probands with PDD/autism were assessed using the FHI (total: 1327 BR and 326 NBR). Individual FHI items showing differences between BR and NBR were entered into a factor analysis. Factor scores were calculated for male and female relatives, and for subgroups of relatives defined by proband characteristics (IQ>70 vs IQ<70, and single vs multiple incidence family). Results: 23 out of 39 FHI items significantly discriminated BR from NBR. Factor analysis (with Varimax rotation) identified 7 orthogonal factors: 1)Social isolation; 2)Learning problems; 3)Social-pragmatic; 4)Odd/inappropriate behaviour; 5)Rigidity; 6)Quality of adult relationships; and 7)Speech delay/deviance. These factors showed generally higher relative risks to BR vs. NBR than the three components of the existing algorithm. Each factor discriminates male BR from male NBR. Female BR, compared to female NBR, score higher on all factors except 4). Analysis using the proposed 7-factor structure yields both qualitative and quantitative differences between BR and NBR of high vs. low IQ probands and in single vs. multiple incidence families. The subset of factors with higher scores in BR than NBR differs by proband characteristics; as well, there are differences between BR of proband subgroups.

Conclusions: These results suggest that the BAP may be comprised of up to 7 genetically informative traits. Differences between BR of proband sibgroups suggest that at least some of these traits may be governed by different genetic mechanisms.

Risk factors for liver disease in cystic fibrosis (CF). *A.S. Rigby¹, O.M. Pirzada¹, C.J. Taylor¹, J. McGaw¹, S. Evans², M.S. Tanner¹.* 1) Sheffield Children's Hosp, Univ Sheffield, Sheffield, UK; 2) Centre for Human Genetics, Univ Sheffield, Sheffield, UK.

Life expectancy in CF has increased significantly over the last decade. With better survival non-respiratory manifestations such as liver disease are becoming more important. The aim of this study was to determine the prevalence of and risk factors for liver disease in CF (CFLD). Case notes and annual review records for all 164 patients (median age 9.4 years) registered at the Sheffield Children's Hospital over a one year period were reviewed. All patients had undergone repeated abdominal examination by CF physicians and had been through our annual review protocol. Risk factors included age, gender, pancreatic sufficiency, portal hypertension, CFTR genotype, neonatal meconium ileus, raised transaminases, gall bladder abnormalities and the Westaby score (severity of ultrasound findings). Statistical associations were calculated by odds ratios (ORs) with 95% confidence intervals (CIs). The RPE method was used to calculate allele differences. Clinical evidence of liver disease was present in 28 (17%) patients, 60% of whom had portal hypertension. There was a peak incidence in adolescence. No patient with CFLD was pancreatic sufficient. 79 (59%) of all patients were homozygous for $\Delta F508$. Three variables were significantly associated with liver disease after adjusting for possible confounding in a multiple logistic regression model; pancreatic insufficiency, raised transaminases (OR=18.7, 95% CI=1.4-250) and the Westaby score. Patients with Westaby scores ≥ 7 were 66 times as likely to have liver disease compared to patients with scores ≤ 3 ; a dose-response effect was evident. There were no significant associations with gender or neonatal meconium ileus. A separate analysis based on patient alleles found an excess of 1138insG (observed=3, expected=0.4, Chi-square=16.4, $p<0.01$). Liver disease and portal hypertension are more common in CF than previously reported. There was an excess of 1138insG, a frameshift mutation in exon 7, compared to the CF population in Europe ($<0.4\%$ of CF chromosomes). The biological significance of this needs further evaluation.

Familial Aggregation for Traits Associated with Insulin Resistance: The IRAS Family Study. *L. Henkin¹, W.M. Brown¹, J.E. Hokanson², S.S. Rich¹*. 1) Wake Forest University, Winston-Salem, NC; 2) University of Colorado, Denver, CO.

Insulin resistance (IR), a feature of Type 2 diabetes, is a cardiovascular risk factor acting through hypertension, dyslipidemia, impaired fibrinolysis, visceral adiposity, and direct atherogenic effects of insulin. Familial aggregation of components of IR has been demonstrated, suggesting genetic regulation of these factors. The IRAS Family Study proposes to identify genes for IR and their interaction with environmental factors in Hispanic and African American families. Here we report preliminary heritability estimates for factors related to IR in the initial 50 pedigrees.

The IRAS Family Study is recruiting 1440+ members from 160 extended families in San Antonio, TX, San Luis Valley, CO, and Los Angeles, CA. Phenotypic data include insulin resistance by frequency sampled intravenous glucose tolerance (FSIGT), anthropometry, visceral adiposity by computed tomography (CT), blood pressure, lipids, lipoproteins, PAI1, fibrinogen, albumin, creatinine, and health behavior interviews. DNA is collected for a genome screen. A variance components method (SOLAR) is used to estimate heritability of BMI, insulin, glucose and the HOMA index (a measure of insulin resistance). Analyses include age, gender, ethnicity, and diabetic status as covariates.

The mean \pm SD age for the subjects with clinical data is 48 \pm 16.0 years. In diabetics, the mean BMI is 31.9 \pm 8.1 kg/m², fasting insulin 28.2 \pm 26.3 mU/ml, glucose 156 \pm 48.9 mg/dl, and HOMA 28.7 \pm 26.6. In non-diabetics, the mean BMI is 28.3 \pm 7.3 kg.m², fasting insulin 14.4 \pm 13.3 mU/ml, glucose 96 \pm 17.2 mg/dl and HOMA 10.6 \pm 11.1. Heritability is estimated for BMI as 0.54 \pm 0.12 (p<.01), HOMA as 0.39 \pm 0.12 (p <.01), insulin as 0.22 \pm 0.11 (p<.01), and glucose as 0.08 \pm 0.08 (p=.12). Thus, there is evidence for significant genetic influences on insulin resistance, fasting insulin and BMI, but no significant evidence of heritability of fasting glucose after controlling for diabetic status. These results suggest that the IRAS Family Study pedigrees are useful in identifying genetic determinants of insulin resistance and related phenotypes.

Power of Omnibus Likelihood Ratio Test for Haplotype-based Case-control Studies. *D. Fallin*¹, *N. Schork*^{1,2,3,4}. 1) Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Statistical Genomics, Genset Corp., La Jolla, CA; 3) Biostatistics, Harvard University, Boston, MA; 4) Jackson Laboratories, Bar Harbor, ME.

Haplotype-based analyses may have increased power over single-locus tests given their ability to capture unique chromosomal patterns. The use of haplotype-based tests for association analyses has been difficult, however, given the need for phase information. In TDT analyses, this is partly overcome through the use of family information, although many trios are still uninformative and therefore not useful. The situation is even worse in case-control analyses between unrelated individuals, where only complete, or singly heterozygous individuals are informative. We have developed a testing procedure for group comparisons that uses haplotype estimation via the E-M algorithm to predict phase in ambiguous individuals and to test differences between haplotype frequencies profiles of case and control groups. In this presentation, we demonstrate the power of such an approach compared to single locus analyses, and under several different alternative hypothesis scenarios. We show that unless the disease allele itself is included in the marker set, haplotype analysis will always be more powerful than testing single loci. We also show the power of our omnibus approach compared to other haplotype-based methods when more than one haplotype for the same gene region is associated with disease risk.

Large-scale empirical assessment of the properties of haplotype-based tests of genetic association. *L. Essioux¹, R. Norman³, N. Tahri¹, T. Chu³, S. Lissarrague¹, D. Fallin², C. Curtis³, B. Skierczynski³, N.J. Schork³.* 1) Statistical Genomics Department, Genset, Paris, France; 2) Dept of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH; 3) Genset Corp. LaJolla, Ca.

Haplotype analyses have become more and more popular as genotyping costs have decreased and as the number of available genetic markers has increased. Although several different strategies for haplotype analysis have been proposed, all of them depend critically on the amount of linkage disequilibrium (LD) between alleles at loci within a genomic region of interest. This is especially the case when estimated haplotype frequencies are used in a case/control analysis setting. Modeling LD when assessing the power of haplotype analyses in such settings often involves arbitrary assumptions about its strength and the distances over which it can be found. As an alternative to making potentially unrealistic assumptions of LD strength, we designed a study of the properties of haplotype analyses, which makes use of observed haplotype frequencies in a sample of Caucasians. This sample consisted of over 200 French blood donors genotyped on over 50 markers in two fully sequenced genomic regions of over 150 kilobases in length. The empirically derived distribution of LD and haplotype frequencies observed in this sample was then used to guide power studies of different haplotype-based association analyses. Our results help put into perspective the likely yield of high-density SNP mapping initiatives for many diseases.

Computational and algorithmic improvements in expectation/maximization (EM) algorithm-based haplotype analyses. *S. Lissarrague, L. Essioux, N.J. Schork.* Statistical Genomics Dept, Genset Corp., La Jolla, CA.

Technological advances have greatly increased the number of applications associated with Human Genome Initiative and related initiatives. Foremost among these applications is the identification of disease genes using high-density maps of single nucleotide polymorphisms (SNPs). There are a number of issues, which plague the use of high-density maps for disease gene identification, however. Most of these issues have to do with way in which one can usefully extract information from relevant genotyping assays. The Expectation/Maximization (EM) algorithm can be used to estimate haplotype frequencies from high-density multilocus genotype data and therefore can be of great utility in gene mapping studies. We describe improvements in the EM algorithm for use in haplotype analyses involving biallelic markers. The improvements are implemented at the design, implementation, and computational levels of the algorithm and include recoding relevant items as a system of binary variables and the use of parallel computing architectures. We present several comparisons between different computational strategies and our own and consider CPU time studies as well as other issues. Our studies show great promise as computational advances in modern genomic analyses.

Epidemiology of congenital malformations in Belarus and Chernobyl accident. *G.I. Lazjuk, D.I. Nikolaev, R.D. Khmel.* Department of Monitoring for Congenital Malformation, Institute for Hereditary Disease, Minsk, Republic of Belarus.

At the 48th Congress of the American Geneticists (Baltimore, 1997) we reported the correlation between the prevalence of congenital malformations (CM) and Cs¹³⁷ contamination density of the areas. For 2 subsequent years (1998 and 1999) the situation remained unchanged. The prevalence of CMs in the areas with Cs¹³⁷ contamination density of 555 kBq/m² (15 Ci/km²) was registered as 7.29/1000, and in the areas with contamination density of 1.0 Ci/km² it was recorded as 7.05/1000.

However, the study of possible teratogenic efficiency of collective and individual doses for the population of these areas has found no dependence of the doses and CM prevalence. Per 1% increase of CM prevalence in Gomel region there was 155 p/Sv, and in Mogilev region it was 78 p/Sv. Similarly, 1.0 mSv of additional exposure dose in Gomel region corresponds to 3 - 6.6/1000 of CM increase, and 1.0 additional mSv in Mogilev region corresponds to 4.2 - 9.7/1000 increase. Teratogenic efficiency of equal in their composition radionuclides from both regions should be equal. The differences we found allowed us to think that increased prevalence of CM is due to not only additional exposure from Chernobyl, but also due to additional factors various in Gomel and Mogilev regions, such as various levels of soluble selen salts and stable iodine in these regions.

Mutations on the CYP21 gene and haplotype analysis of steroid 21-hydroxylase deficiency alleles in Brazil. *M.P.*

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Congenital adrenal hyperplasia (CAH) due to steroid 21-hydroxylase deficiency is a common inherited defect of adrenal steroid hormone biosynthesis. Unusually for a genetic disorder, the majority of mutations causing CAH result from recombinations between the CYP21 gene encoding the 21-hydroxylase enzyme and the closely linked, highly homologous pseudogene CYP21P. The CYP21 and CYP21P genes are located in the major histocompatibility complex class III region on chromosome 6p21.3, a region that undergoes high recombination rates. Studies on the molecular basis of steroid 21-hydroxylase deficiency in Brazil have revealed the presence of fourteen mutations distributed among 117 chromosomes. The percentages of individual mutations does not differ from other findings in different populations. We have performed haplotype analysis on a total of 111 chromosomes using Taq I CYP21 and C4 RFLP/Southern blotting, mutation-specific PCR and PCR/RFLP for two intragenic polymorphisms in order to study the chromosomal background of ten recurrent mutations and four new mutations. Fifty four different haplotypes were identified. The number of haplotypes varied from five to nine for each the five most frequent mutations. These data reflect the wide heterogeneity of the Brazilian population, and show that most recurrent mutations on the CYP21 gene are of various independent origin. Supported by Grants from: CAPES, CNPq and FAPESP.

Self-reported health history survey (Q8) and genetic analyses in the NAS-NRC aging twin panel cohort. *T. Reed¹, J.C. Christian¹, W.H. Page².* 1) Dept Medical & Molecular Genetics, Indiana Univ Medical Ctr, Indianapolis, IN; 2) Medical Follow-Up Agency, National Academy of Sciences Washington, DC.

Purpose: To survey an elderly cohort of male twins for various health outcomes, estimate the heritability (h^2) of the traits, and based on the frequency of occurrence to determine the feasibility of obtaining DNA for linkage studies.

Methods: The National Academy of Sciences-National Research Council World War II veteran twin registry is comprised of white male twins born between 1917-27. An epidemiologic questionnaire, Q8, contained new items about the twins' health history and repeated some items from two prior surveys concerning alcohol consumption, cigarette smoking, cardiovascular disease symptoms, hypertension, diabetes, stroke, liver cirrhosis and cataracts. Q8 was mailed between Sept-Nov 1998 to 4086 pairs with known addresses and to 908 men who had completed both of the prior surveys, but whose brother had died. Eliminating surveys returned because of bad addresses or death of the subject, 6104 of 8848 (69%) completed forms were received comprising 2058 complete pairs and 1988 singletons with a mean age of 74 years.

Results: Genetic analyses were undertaken using complete pairs for self-reported presence or absence of disease, including calculation of probandwise concordance rates and h^2 estimates with the program Mx. A trait of "wellness" (defined as no heart attack, coronary surgery or angioplasty, stroke, diabetes, prostate cancer, or hypertension) had a concordance rate in MZ pairs (0.59) that was significantly higher than the concordance rate in DZ pairs (0.41). The best fitting model for wellness was one which included additive genetic effects and no common environmental effects with a h^2 estimate of 0.57 (95% confidence interval, 0.49-0.64). Migraine, using the International Headache Society classification, had a h^2 estimate of 0.34 (0.11-0.54), but too few concordant or discordant pairs to pursue linkage studies. Presbycusis, hearing loss with age, had a h^2 of 0.61 (0.54-0.68).

Mapping of genes for alopecia areata. *M. Barsasteanu*¹, *D. Papadopoulos*¹, *J. Fasolo*¹, *K. Ronningen*², *A. Sinha*¹, *S. Iyengar*³. 1) Dermatology, Weill Med College, Cornell U, New York, NY; 2) National Institute of Public Health, Oslo, Norway; 3) Dept of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH.

Alopecia Areata (AA) is an autoimmune disease in which anagen hair follicles become the target of immune cell attack, with 0.05%- 0.1% of the population being affected at any one time. The cloning of the recessive gene for alopecia universalis (AU) on human 8p12 from a consanguineous family led us to hypothesize that genes for AA may segregate differently in smaller families with at least two affecteds. National Alopecia Areata Foundation (NAAF) investigational guidelines were used to define alopecia. Our investigation was limited to three forms of alopecia: patchy AA, Alopecia Totalis (AT), and AU. Patients were screened by personal interview and medical record review for a history of scalp hair loss, body hair loss, nail involvement and family history of AA, AT or AU. Of 142 AA patients, 118 (79%) consented to participate. Seventy seven percent of the patients had AA, 20% had AT/AU and 3% had AT. The racial composition was 17% African American, 56% Caucasian, 21% Hispanic and 6% Asian and was not significantly different between index cases with and without a family history. Thirty two percent of probands had a positive family history. The mean age-at-onset of the probands was 30 years. DNA from probands with and without family history of AA was typed for polymorphisms at the HLA-D locus. HLA-DR4 and DR-11 as well as HLA-DQ*03 alleles were found to have an increased frequency in cases versus controls. Due to racial variation in allele frequencies at the HLA loci, all comparisons were race-specific. Our data suggest that AA aggregates in families with possible involvement of HLA loci in AA pathogenesis. Genotyping at additional candidate genes (eg. AU locus) in affected relative pairs with AA is in progress and should facilitate identification of genes controlling AA susceptibility.

The familial aggregation of age-of-onset of childhood-onset type-1 diabetes in a population-based diabetes register from Western Australia. *L.J. Palmer^{1,2}, T.W. Jones³, E.A. Davies³, P.R. Burton⁴*. 1) Dept Epidemiology & Biostat, Case Western Reserve Univ, Cleveland, OH; 2) Channing Laboratory, Brigham and Women's Hospital and Harvard University, Boston, MA; 3) Dept Endocrinology, Princess Margaret Hospital for Children, Perth, Australia; 4) Dept Epidemiology and Public Health, University of Leicester, Leicester, UK.

Insulin dependent diabetes mellitus (IDDM) is known to be under a high degree of genetic determination. However, it is currently unknown if age-of-onset of childhood-onset IDDM aggregates within families, and if so to what extent such aggregation may be due to genetic factors. Our aim was to investigate the nature of any such familial aggregation in a population- and family-based register of all incident cases of childhood (<18yrs) IDDM in Western Australia. Gibbs sampler models (BUGS) were used to perform complex variance components analysis on the right-censored trait age-of-onset. Analyses assumed a Poisson link and a proportional hazards survival model, and were adjusted for ascertainment and for potentially confounding covariates. The dataset contained 869 index cases, 869 nuclear families and a total of 4,033 subjects. Median age-of-onset of IDDM was 7.9 years (range=0.8 to 17.8 years). The ls of childhood onset IDDM was approximately 20. BUGS analyses suggested IDDM age-of-onset comprised additive genetic components of variance ($s^2A=7.23$, 95%CI=1.20 to 23.05), little or no familial environmental components ($s^2C=0.00$), and common sibling environmental components ($s^2CS=3.41$, 95%CI=0.73 to 10.07). These data are consistent with the existence of a substantial degree of familial aggregation of age-of-onset of IDDM, the majority of which is due to additive genetic factors, and raise the possibility that childhood-onset IDDM (ls20) may be under stronger genetic control than adult-onset IDDM (ls15). Programs of gene identification in IDDM may be enhanced by the study of childhood-onset IDDM and by the use of age-of-onset as a phenotype.

Familial Aggregation and Segregation Analysis of Some Blood Pressure Related Phenotypes in A Rural U.S.

Caucasian Community. *S.K. Nath*¹, *A. Chakravarti*², *R. Cooper*³, *A. Weder*⁴, *N.J. Schork*¹. 1) Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Department of Genetics, Case Western Reserve University, Cleveland, OH; 3) Department of Preventive Medicine, Loyola University, Chicago, IL; 4) Department of Medicine, University of Michigan Medical School, Ann Arbor, MI.

Familial correlation and segregation analyses of some blood pressure and obesity related phenotypes i.e., systolic blood pressure, diastolic blood pressure, body mass index etc. were performed to assess their major genetic determinants. In order to evaluate the pattern of familiarity and major gene segregation in these traits, we used the nuclear family data ascertained through a proband from a rural US Caucasian community (Tecumseh, MI). Each phenotype was adjusted for covariates, i.e., age and sex. In addition, all the phenotypes were transformed to approximate normality via Box/Cox power transformation. We used class D regressive models for segregation analyses. The pattern of familial correlation and estimated segregation parameters of some phenotypes show the involvement of major gene. We discuss the implications of our findings for gene discovery initiatives. The data for this study were generated by GenNet Network of the Family Blood Pressure Program sponsored by the National Heart, Lung and Blood Institute (HL 54998-01).

Mitochondrial DNA variability in Turkic-speaking populations of the Altai and Sayan region from South Siberia.

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It is well established by anthropologists and archaeologists that the territories of South Siberia were the ultimate source of important population expansions into Eurasia. Being inhabited since Upper Paleolithic those territories act as an interaction zone between Caucasoid and Mongoloid tribes. To investigate the origins and evolution of South Siberian populations we present here the mitochondrial DNA (mtDNA) variability data in five Turkic-speaking groups of the Altai and Sayan region. The key RFLP mutations defining the main Eurasian mtDNA haplogroups (A, B, C, D, E, H, U, T, J) were analyzed in a sample of 262 individuals: the Altai-Kizhi, Khakassians, Sojots, Tuvinians and Shorians. This analysis showed that the majority of the Tuvinian and Sojot mtDNAs (86,2% and 88,2%, respectively) belong to haplogroups A, B, C, D, E, and M* characteristic for Mongoloid populations. However, only 47,8% of the Altaians and 53,7% of the Khakassians share mtDNAs from those typically Mongoloid haplogroups. Very limited Mongoloid influence (19,1%) has been found in the mitochondrial gene pool of Shorians. 9-bp deletion in COII-tRNA(Lys) mtDNA intergenic region was found in all groups studied with the frequencies varying from 13,9% in Tuvinians to 2,4% in Shorians. Caucasoid-specific haplogroups (H, U, T, J) were identified in 35,7% of the Shorians, 22,8% of the Altaians, 18,5% of the Khakassians, 5,9% of the Sojots and 5% of the Tuvinians, with haplogroup U mtDNAs observed in all populations. Despite the same linguistic affinities, the populations studied differ considerably in the pattern of mtDNA haplogroups distributions, suggesting thereby the dual origin for Altaians, Khakassians and Shorians, whereas the Tuvinians and Sojots appear to be the typical Central Asian Mongoloids. Work supported by RFBR grants 99-06-80430 (MD), 00-06-80374 (ID).

Y-chromosome haplotypes demonstrate the presence of paleo-Caucasoid component in Khakassians. G.

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The Y-chromosomes of Turkic-speaking Khakassians (aboriginal ethnic group inhabiting the plains of the Minusin Basin of Altai and Sayan region) were analyzed with a set of five biallelic polymorphisms identifying variable loci in the nonrecombining portion of the Y chromosome. The analysis revealed the presence of two major haplotypes in male pool of Khakassians. The most frequent haplotype associated with 92R7 HindIII(-) allele, SRY1532 A allele, as well as with the ancestral states of the polymorphisms YAP, TAT, and DYS199, was found in 14,3% of Khakassians. The Y-chromosome haplotype, differing from one described above only by the mutation at SRY1532, was very frequent (10,2%) in Khakassian sample. Both of haplotypes were found in association with 92R7 allele, which defines at least seven haplotypes, that are important for the tracing of the major migrant Y-chromosome to the Americas (Santos et al., 1999). The Y-chromosome haplotypes found in Khakassians are identical apparently to haplotypes 20 and 32, derived from haplotype 10 (Santos et al., 1999), which is a common ancestor between Native Americans and Europeans, who left some rare descendants in Siberia, among the Kets and Altaians. Moreover, our preliminary data indicate that 92R7-derived Y-chromosome lineages widely distributed in the region of Altai and Sayan Mountains being also found in South Altaians, Tuvinians, and Sojots. Overall, the data received are in accordance with the paleoanthropological views (Alekseev, 1989) suggesting that the tribes with the Caucasoid morphological features were prevalent in Altai and Sayan region since Neolithic and Bronze Age. Their subsequent replacement by Central Asian and North Asian tribes led to formation of population with typical Mongoloid morphological traits. Work supported by RFBR grants 99-06-80430 (MD), 00-06-80374 (ID).

MITOCHONDRIAL DNA VARIATION IN RUSSIANS, UKRAINIANS AND IRANIANS. *B. Malyarchuk¹, G. Denisova¹, M. Derenko¹, I. Zakharov²*. 1) Genetics Lab., Institute of Biological Probl. of the North, Magadan, Russia; 2) Genetics Lab., Vavilov Institute of General Genetics, Moscow, Russia.

Mitochondrial DNA (mtDNA) polymorphism in 50 Russians and 38 Ukrainians was examined by RFLP analysis and hypervariable segment I (HVS I) sequencing. Together, the Russians and Ukrainians were found to have mtDNAs belonging to nine haplogroups (H, V, T, J, U, K, I, W, X) observed in all European populations. The data received were combined with those available for other European populations and subjected to analysis of shared haplotypes distribution. It was found that the Russians and Ukrainians exhibited a high portion of identical or similar haplotypes in relation to those observed in other Europeans. On the level of rare haplotypes, the Russians were more closely linked to German and Finno-Ugric populations. For instance, subhaplogroup H1 mtDNAs were found in common only between Russians and German-speaking populations. It has been proposed by archaeologists and anthropologists (Alekseeva, 1973; Sedov, 1979), that modern Ukrainians were formed on the basis of Iranic tribes inhabited the Pontic steppes and Slavonic tribes who expanded there from Central Europe approximately 1500 years ago. To estimate the possible Iranic contribution in the gene pool of the Ukrainians, mtDNA variation of 25 Iranians collected from various regions of Iran was examined by RFLP analysis. It was found that Iranian mitochondrial gene pool is represented by West Eurasian haplogroups T (12%), J (12%), U (16%), K (4%), W (12%), I (4%) and additional lineages. However, haplogroup H, which encompasses 40% of the Ukrainian mtDNAs, is virtually absent in Iranians, as well as in Indians. The East Asian-specific macrohaplogroup M sequences were observed in 8% of Iranians. In addition, 9-bp deletion in region V on the background of +10394DdeI/-10397AluI and -10394DdeI/-10397AluI haplotypes was found in 8% of Iranians studied. The data received allow us to conclude that the modern Ukrainians appear to be closer to the European populations, than to West Asian ones. This work was supported by the Russian Fund for Basic Research (grant 00-06-80448).

Conserved worldwide linkage disequilibrium in the human factor XI gene. *S.M. Williams¹, T. Tarumi², D. Martincic², J.A. Whitlock², J.H. Addy³, D. Gailani².* 1) Dept Microbiology, Meharry Medical Col, Nashville, TN; 2) Vanderbilt University Medical Center, Nashville TN; 3) University of Ghana Medical School, Accra, Ghana.

Factor XI is a plasma coagulation protease, the deficiency of which causes a bleeding disorder characterized by excessive post-traumatic bleeding. We evaluated the exons of the human factor XI gene that code for the mature protein (exons 3-15) to determine the frequencies of common single nucleotide polymorphisms (SNPs) and haplotype distributions. A total of 266 F11 alleles were screened from 41 Caucasians, 50 Ghanaians, and 42 East Asians (Japanese, Chinese, and Korean). Eleven SNPs were identified, four of which were in flanking non-coding regions. Only five SNPs (all coding or cSNPs) had minor allele frequencies >5% in at least one population; none of these five encoded an amino acid change. The cSNPs are all in Hardy-Weinberg equilibrium, and all cSNPs are present in the three populations (with one exception in East Asians), indicating ancient origins. Three of the cSNPs, C472T, A844G, and T1234C, that are spread across ten kilobases of DNA are in significant LD with each other. In all three populations 844G and 1234C are always found together, with the exception of one Ghanaian. Furthermore, 472T is always associated with 844G-1234C. Estimated haplotype frequencies from all three populations are significantly different from expected ($p < 10^{-4}$). In contrast, the other cSNPs, C1750T and G1855T, are in equilibrium with each other and the three other cSNPs. We analyzed 40 additional individuals of East-African origin (Ethiopian Jews) and found a similar LD pattern. Our data demonstrate marked LD in the human F11 gene, and show that haplotype patterns are strikingly similar across populations, despite significantly different allele frequencies. Given the apparent old age of the 472T-844G-1234C haplotype, the distances between the nucleotide residues, and the fact that other cSNPs in this gene that are close together are in linkage equilibrium suggests an interesting evolutionary history for these polymorphisms.

mtDNA diversity of Colombian Amerind populations. *M.M Torres¹, D. Ortiz-Barrientos², I.D. Soto², L.G Carvajal³, C. Duque², G. Bedoya², H. Groot de Restrepo¹, A. Ruiz-Linares^{2,3}.* 1) Laboratorio de Genetica Humana, Universidad de los Andes, Bogota, Colombia; 2) Laboratorio de Genetica Molecular, Facultad de Medicina Universidad de Antioquia, Medellin, Colombia; 3) Department of Medical and Community Genetics, Imperial College School of Medicine, UK.

In order to examine mtDNA diversity in Colombian Amerinds we performed a combined RFLP and D-loop sequence analysis of 73 individuals from 17 Native populations. In each sample we collected sequence data for D-loop hypervariable segments 1 and 2 and typed 5 RFLPs (the 4 sites diagnostic of lineages A to D and the HaeIII site at position 16,517). The populations examined included representatives of the 4 main linguistic South American subdivisions: Chibchan-Paezan, Ge-Gano-Caribe, Andean and Equatorial-Tucano. We identified in this sample sixty seven sequence haplotypes all carriers of most of the changes characteristic of founder mtDNA lineages A, B, C and D. These data question the existence of some proposed additional founder lineages (A2; X6 and X7). Analysis of sequence diversity across main South Amerind linguistic divisions indicates significant genetic structure and is in qualitative agreement with the linguistic classification and the geographic distribution of the populations examined. We detected evidence of recombination in mtDNA as indicated by a significant correlation between strength of linkage disequilibrium and physical distance between polymorphic sites.

Mitochondrial D-loop sequences and genetic diversity of Chinese ethnic populations. *Y-P. Zhang¹, Y. Yao¹, H. Harpending², Y-X. Fu³.* 1) Lab, Cellular & Molecular Evol, Kunming Institute of Zoology, Kunming, 650223, People's Republic of China; 2) Department of Anthropology, University of Utah, Salt Lake City, UT 84112; 3) Human Genetics Center, University of Texas - Houston, Houston, Texas 77030.

Mitochondrial DNA control region segment I sequences were examined in 261 individuals from 8 Chinese ethnic populations and 32 Thais. 206 haplotypes were typed, confirming the high variability of the region. Both mismatch and phylogenetic analyses suggested that the marked distinction between the southern and northern Chinese ethnic groups was due to their earlier origin and differentiation and the southern groups were more ancient. Assuming a divergence rate of 33% site/myr (Ward et al. 1991), we estimated the divergence time of the southern and northern groups to be about 60,000 years before present. Linguistic and geographic classifications of the Chinese populations were not strictly reflected in their mtDNA variation. Mismatch and neutrality test analyses suggested that Chinese ethnic populations could have undergone expansions in the past, with the exception of Tibetan and Chinese Mongolian of which might have different demographic reasons. Combined with the analyses of other world populations, we tentatively propose that Southeast Asia or southwest China might be the place where subsequent human expansions originated after the initial out-of-Africa expansion.

Evolution of the mtDNA 9-bp deletion in East and Southeast Asia populations. *Y.Y. Shugart¹, W.S. Watkins², Y-P. Zhang¹*. 1) Lab Cellular & Mol Evol, Kunming Institute of Zoology, Kunming, Yunnan 650223, China; 2) Department of Human Genetics, University of Utah, Salt Lake City, Utah 84112.

1218 Chinese from 12 ethnic groups and 9 Han geographic groups were screened for the mtDNA 9-bp deletion motif in the COII/tRNALys intergenic region using PCR-PAGE. The frequency of the 9-bp deletion in all samples was 14.7%, but ranged from 0% to 32% in different ethnic groups. There is a general decrease tendency in the deletion frequencies from the south to the north, from coastal to inland. Additionally, 3 individuals were confirmed to have a triplication of 9-bp segment. Phylogenetic and demographic analyses of the mtDNA hypervariable segment 1 (HVS1) sequences suggests that the 9-bp deletion occurred more than once in China. The majority of the Chinese deletion haplotypes (90%) have a common origin that originated from a mutational event following an initial expansion of modern humans in eastern Asia. Other deletion haplotypes and the three haplotypes with a 9-bp triplication, may have arisen independently in the Chinese, presumably due to replication error. Two possible migration routes of the 9-bp deletion in east and Southeast Asia, both were originated in China, with one route leading to the Pacific Islands via Taiwan, the other to Southeast Asia and possibly the Nicobar Islands are estimated from mtDNA HVS1 analysis. Along both routes of peopling, a decrease in HVS1 diversity of the mtDNA haplotypes with the deletion was observed. The "Polynesian motif" and the 16140 T/C, 16266 C/A or C/G polymorphisms appear specific to the each migration route, respectively.

Melanocortin-1 receptor gene mutations and polymorphism in Chinese ethnic populations. *X-M. Lu, P. Shi, H-R. Luo, Y-P. Zhang.* Lab Cellular & Molecular Evolution, Kunming Institute of Zoology, Kunming, Yunnan 650223, China.

Melanocortin-1 receptor (MC1R) is a seven transmembrane domain G protein-coupled receptor of 317 amino acids that belonging to the melanocortin receptor subfamily and has high binding affinity for MSH and ACTH. Previous studies have shown that MC1R is highly polymorphic in European and Asian populations, and that there are strong correlation between MC1R variants and the color of human hair and skin. Here we examined the polymorphism of MC1R gene in 438 individuals from 16 Chinese ethnic populations. Both PCR-SSCP (single-strand conformation polymorphism) and direct sequencing were used to identify the MC1R variants. Besides the three common variants (Val92Met, Arg163Gln and A942G), we also detected 10 rare variants (Val60Leu, Arg67Gln/Arg163, Ala167Glv, His216Val, Pro230Leu, Asn291Lys, Ala299Val, C478T, C414T and C645T) in total samples. For variant Ala167Glv, His216Val, Pro230Leu, Asn291Lys, Ala299Val, C478T, C414T and C645T, they were reported for the first time. The frequency of the Arg163Gln variant was variable in different ethnic groups, ranged from 85.0% (Tibetan) to 40% (Uygur), while those of Val92Met and A942G are not greatly more or less than 12.5%, with the exception of 31.8% in Dai and 38.5% in BuYi. Positive Darwinian selection on MC1R gene, as well as the random genetic drift, migration and the admixture of various ethnic groups might have account for the frequency distribution of the MC1R variant in different ethnic populations.

Caste-and tribe-specific Y-chromosome haplotypes based on Single Nucleotide Polymorphisms in seven endogamous population groups of Andhra Pradesh, South India. *G.V. Ramana¹, B. Su¹, L. Jin¹, L. Singh², P. Underhill³, R. Chakraborty¹.* 1) Human Genetics Center, University of Texas, Houston, TX; 2) Center for Cellular and Molecular Biology, Hyderabad, India; 3) Department of Genetics, Stanford University, Stanford, CA.

It is now well established that Single Nucleotide Polymorphism(SNP)markers on the Y-chromosome reveal population-specific Y-haplotypes that are useful in tracing past population movements. Consequently, Y-SNP haplotypes can be used to detect male gene flow across populations. To study such utility of Y-chromosome genetic variation, we have analysed 26 Y-SNP markers in 204 males from Andhra Pradesh, South India. The individuals studied belong to 3 caste groups (Vizag Brahmins, Peruru Brahmins, Kammas), 3 tribes (Bagata, Poroja, Valmiki), and an additional group (the Siddis) of African ancestry. We have identified 11 distinct haplotypes in the total sample. Haplotypes (H5 and H14) that are present in Southeast Asia, Europe and Oceania are shared by all of the groups studied. The H4B haplotype, a derivative of the M52 mutation, is also present in all groups except the Siddis. The tribal groups lack the haplotypes H4, H4A, H5A, and H16, which are seen in the caste groups. In contrast, they exhibit the Southeast Asian haplotype H11 that is absent in the caste populations. The Valmikis have an intermediate position, in the sense that they exhibit 9 of the 11 haplotypes. The presence of haplotypes H4, H5, H14, and H16 in the Siddis indicate that they have assimilated considerable non-African admixture. The principal component analysis supports these observations, placing the Siddis, the three caste populations, and the two tribal groups in three clusters, with the Valmikis closer to the caste groups. In summary, even though these SNP-based Y-haplotypes are able to distinguish the populations, the present data indicates that male gene flow in these South Indian populations is not as negligible as that reflected from the study of Y-specific short tandem repeat markers. (Research supported by the US Public Health Service Research Grant GM 41399 from the National Institutes of Health).

Genetic structure of world populations using polymorphic Alu retrotransposons. *W.S. Watkins¹, C.E. Ricker¹, M.J. Bamshad^{1,2}, H.C. Harpending³, A. Rogers³, S.V. Nguyen⁴, M.L. Carroll⁴, M. Batzer⁴, L.B. Jorde¹.* 1) Department of Human Genetics, University of Utah, Salt Lake City, UT 84112; 2) Department of Pediatrics, University of Utah, Salt Lake City, UT 84112; 3) Department of Anthropology, University of Utah, Salt Lake City, UT 84112; 4) Department of Pathology, Biometry and Genetics, Biochemistry, and Molecular biology, Louisiana State University Medical Center, New Orleans, LA 70112.

Retrotransposition of Alu elements produces new polymorphic loci that are ideal for human genetic analyses because the ancestral state of each locus is known. We have ascertained 38 unlinked, human-specific polymorphic Alu loci and have typed 715 individuals from 31 world populations using these markers. African populations have higher Alu gene diversity (0.34) than Asian (0.25), European (0.22), and Indian (0.25) groups. A neighbor-joining tree based on Alu insertions unambiguously separates African populations from other populations (bootstrap support = 100%). The root of the tree is located among sub-Saharan African populations. Principal-components plots based on Alu genetic distances also show continental clustering, and African populations are closest to the ancestral state. Alu genetic distances show high and significant ($p < 0.0001$) Mantel correlations with other genetic markers (Alu-RSP: 0.875, Alu-STR: 0.770, Alu-mtDNA: 0.796) in 15 populations typed for all systems. The fixation index (F_{st}), indicating the diversity attributable to population subdivision, is 16% and similar to that of other nuclear markers. Alu/Alu observed homozygosity is lowest in Africa (0.25) and is significantly higher for Asian (0.45), European (0.44), and Indian (0.42) groups. The genetic distance of each population to the regional centroid indicates higher than expected heterozygosity and gene flow for African populations and expected to lower than expected heterozygosity for most non-African subpopulations.

Origin of Haplogroup M in Ethiopia. *D.C. Wallace^{1,3}, B.P. Donham^{1,3}, T.G. Schurr², D.L. Donham³, C. Panter-Brick⁴, J.T. Lell¹.* 1) Center for Molecular Medicine, Emory University School of Medicine, Atlanta, Georgia 30322; 2) Southwest Foundation for Biomedical Research, Department of Genetics, P.O. Box 760549, San Antonio, Texas 78245-0549; 3) Department of Anthropology, Emory University, Atlanta, Georgia 30322; 4) Department of Anthropology, Durham University, UK.

To elucidate the pattern of genetic variation in East Africa, we characterized the mitochondrial DNA (mtDNA) variation of 157 Ethiopians by restriction fragment length polymorphism (RFLP) and DNA sequence analyses. Ninety-four of these samples were collected in the Maale area of southwest Ethiopia, whereas 61 samples came from northern Ethiopia. In contrast to sub-Saharan Africa from which two-thirds of the mtDNAs belong to haplogroups L1 + L2, and one-third belong to L3; among Ethiopians one-fourth belong to haplogroups L1 + L2, and most of the rest belong to haplogroup L3, including a new L3 mtDNA lineage, present in the Maale. Ethiopians were also found to harbor Eurasian mtDNAs including Asian macrohaplogroup M, defined by the Dde I 10394 and AluI 10397 sites, and European haplotypes H, I, J, T, and U. To determine if Asian M arose in Northeast Africa, we compared the mtDNA sequences of six Asian M mtDNAs with nine African mtDNAs. Three mutations were found at np 14783 T®C, np 15043 G®A, and np 15301 G®A that differentiated the two groups. The 15043 G®A and 15301 G®A mutations were present in L3 and found in all M mtDNAs, but the 14783 T®C mutation was absent in L3, found in half of the Ethiopian M samples, and present in virtually all Asian M mtDNAs studied. Hence, Ethiopian M appears to represent an intermediate between sub-Saharan African and Asian mtDNAs.

The ethnic origins of an enigmatic South Asian population, the Kalasha of northern Pakistan, as revealed by mtDNA variation. *T.G. Schurr¹, W.R. Maggi², K. Fowler³, D.C. Wallace³.* 1) Dept Genetics, SW Foundation Biomedical Res, San Antonio, TX; 2) Department of Anthropology, University of Colorado, Boulder, CO; 3) Center for Molecular Medicine, Emory University School of Medicine, Atlanta, GA.

The origins and affinities of the Kalasha, an enigmatic population from Pakistan's North West Frontier Province, were investigated through the analysis of mtDNA variation. The mtDNAs of ~100 individuals were subjected to both RFLP haplotype and control region (CR) sequence analysis, and the resulting data analyzed with statistical and phylogenetic methods. The RFLP analysis revealed that the Kalasha lacked any mtDNAs which could readily be assigned to haplogroups typically found in southern or northern Asian populations. In fact, they lacked any haplotypes bearing the linked +DdeI 10394 and +AluI 10397 polymorphisms that define macrohaplogroup M in Asian populations. To the contrary, their mtDNAs either belonged to one of the nine haplogroups commonly found in European populations, or else to as yet defined clusters found predominately in South Asian groups. These RFLP data, as well as the CR sequences within them, suggested that the Kalasha lack any genetic affinities with East Asian populations, hence, are not Asian in origin. Instead, they may represent the ancestral Eurasian populations that settled Central Asia prior to the emergence of Indo-Aryan-speaking groups.

Trinucleotide repeat polymorphism analysis in different populations from Russia and nearest countries. S.N.

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Various trinucleotide repeat variability analysis were carried out in different ethnic groups. We investigated CTG and CAG polymorphism associated with some neurodegenerative diseases - myotonic dystrophy (DMPK), dentatorubropalladoluisian atrophy (DRPLA) and spinocerebellar atrophy type 1 (SCA1) - in Slavonic (Russians from Archangelsk, Kostroma, Bellarusians and Ukrainian), Finno-Ugric (Udmurts) and Turk (Khant, Yakut and Komi) populations. Allelic spectrums distinctions were observed among different entice groups for all analysed loci. Statistically significant differences were found for Yakut population comparing with Kostroma (Middle Russian population) by G-statistic ($p=0,0000$). No remarkable distinguishes were observed for Slavonic populations compared with Chant and Komi. We found the tendency for allele distribution spectrums to be varied in South-West and North-East direction, where character of different repeats spectrum change correlating with populations geographic location. Genetic diversity for all populatios were estimated with F-statistic using jackknifing. Fst amount for different population hesitates from 0.016 to 0.041 for DRPLA locus, from 0.005 to 0.21 for SCA1 locus and from 0.005 to 0.024 for MD locus. For all analysed loci Fst quantity for Yakut population were lowest among all populations, suggesting most closeness and genetic distance for this population. Also we constructed genetic tree (using GDA computer program) for investigated populations based on three loci. Phenogram for genetic shows that Yakut population situated far from other population which forms cluster with populations of Caucasian origin having his own relations. Thus two smaller clusters can be obtained first formed by non-Slavonic populations, and second - with mainly Slavonic populations.

Univariate and multivariate analysis of Mexican-American GENNID data. *M.M. Barmada, C.E. Aston.* Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA.

The etiology of type 2 diabetes, though acknowledged to be complex, is still largely unknown. Familial aggregation and concordance in monozygotic and dizygotic twins argue strongly for a genetic component in the etiology of type 2 diabetes. However, studies of putative candidate genes, except in rare subtypes of type 2 diabetes, have not yielded any significant risk factors. We have undertaken an analysis of the phase I Mexican-American data provided as part of the Genetics of Non-Insulin Dependent Diabetes (GENNID) study (Raffel *et al.* 1996). Regression techniques were used to adjust our trait variables (which were derived from the time points of oral glucose tolerance tests) for the effects of several measured covariates. The resulting adjusted quantitative traits were then used to search for quantitative trait loci (QTLs) controlling each trait. Regression models were also developed in a separate, population-based epidemiological cohort from the San Luis Valley Diabetes Study (Hamman *et al.* 1989), and then applied to the GENNID data. Multivariate modeling techniques (principal component and factor analysis) were also used to examine the possibility that there are major genes in diabetes which control susceptibility through pleiotropic action on a number of different quantitative traits. The univariate trait analyses uncovered a number of previously identified type 2 diabetes susceptibility loci, including regions of chromosome 2, 5, 6, 7, 10, and 17. The multivariate trait analyses, though plagued by missing-data issues, still retained enough power to identify similar regions of chromosomes 6 and 17, and also identified new regions of interest on chromosomes 11 and 12.

Raffel LJ, Robbins DC, Norris JM, Boerwinkle E, *et al.* (1996) The GENNID Study. A resource for mapping the genes that cause NIDDM. *Diabetes Care* **19**:864-72

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Analysis of autosomal dominant polycystic kidney disease (ADPKD) in the Newfoundland population. D.S.

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The Newfoundland population is ideal for examining founder effects and for studying the incidence of hereditary disease in what is essentially a closed community. Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common genetic diseases in humans. Mutations in any one of at least three different genes are thought to be responsible for this disease and two genes, PKD1 and PKD2, have been mapped to 16p13.3 and 4q21-q23, respectively. Thirteen Newfoundland families with ADPKD have been examined using microsatellite markers flanking and within the PKD genes on chromosome 16 and chromosome 4. Nine of the thirteen families showed linkage to PKD1 whereas two were linked to PKD2. The other two families were uninformative due to the structure of the family. Comparisons of the haplotypes of the families linked to PKD1 revealed that five families share a partial haplotype suggestive of an ancestral founder disease chromosome. The other four PKD1 haplotypes suggest that these all resulted from independent mutation events. Mutational analysis is currently underway to determine the exact number of founder mutations for ADPKD in the Newfoundland population. (Supported by the Kidney Foundation of Canada and the Medical Research Council of Canada).

Genome scans for quantitative trait loci using variance components linkage analysis: upward bias in heritability estimates attributable to individual quantitative trait loci at lod score peaks. *H.H.H. Goring¹, J.D. Terwilliger², J. Blangero¹*. 1) Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Columbia University and NY State Psychiatric Institute, New York, NY.

While the primary goal of a genome scan is to map the loci underlying the trait of interest, it is also desirable, particularly in the analysis of human diseases and from a public health perspective, to estimate the importance of the identified loci. One such measure of importance is the trait heritability attributable to a mapped locus, i.e. the proportion of overall phenotypic variance in a specific population and environment due to the locus. Variance components-based analyses are widely used to map quantitative trait loci (QTLs). An attractive feature is that these methods not only provide estimates of QTL location, but also of QTL heritability.

Several studies have examined the statistical properties of QTL heritability estimates at a specific point in the genome, such as the location of a known QTL, finding little evidence for systematic bias. We have examined the properties of these estimates in genome-wide scans on simulated data. We have found the QTL heritability estimate associated with the first order statistic, i.e. the highest lod score in the genome, to be biased upwards in general. The explanation is that the locus-specific heritability estimate is positively correlated with the lod score (the heritability estimate associated with a lod score of zero is also zero), such that when the lod score is maximized over the whole genome - containing regions with QTLs as well as many more without QTLs - the QTL-specific heritability estimate associated with the highest lod score peak is likely to be high - often higher than the true heritability of the most "important" QTL. Joint estimation of location and heritability of QTLs is thus complicated by the positive correlation between both measures, such that QTL-specific heritability estimates obtained in genome-wide scans must be interpreted with caution. The same reasoning applies to estimates of locus-specific values of l_s , the relative risk to sibs of an affected individual.

A variance components method of linkage analysis to assess parent-of-origin effects on body mass index. *R.S. Lindsay, S. Kobes, W.C. Knowler, R.L. Hanson. NIDDK, NIH, Phoenix, AZ.*

We have examined the hypothesis that imprinted genes may affect the propensity to obesity. Multipoint linkage to age and sex adjusted body mass index (BMI) was carried out on 846 individuals in 235 nuclear families with 516 autosomal microsatellite markers in a genome wide scan. Parental genotyping, available either directly or by estimation, was then used to calculate identity by descent separately for alleles transmitted from father (p_{FA}) and mother (p_{MO}). Multipoint linkage for BMI was calculated by modification of the variance components method: separate models were examined with (LOD_{IMP}) and without (LOD) parent specific effects and a P value for the difference derived (P_{DIFF}). In addition separate estimates of linkage to paternally (LOD_{FATHER} , using sole effects of p_{FA}) and maternally (LOD_{MOTHER} using sole effects of p_{MO}) derived alleles were obtained. The strongest evidence of linkage was detected on chromosome 11q (at map position 142cM), in keeping with previous analysis of a larger sample of this population (966 individuals, $LOD = 3.6$). No evidence of imprinting effects were found at this site ($LOD = 2.7$, $LOD_{IMP} = 2.1$). Tentative evidence of linkage with significant parent specific effects ($LOD_{IMP} > 1$, $LOD_{IMP} > LOD$ and $P_{DIFF} < 0.05$) was found in two regions. Stronger linkage of BMI to maternally derived alleles was found at 72cM on chromosome 5 ($LOD_{IMP} = 1.2$, $LOD = 0.1$, $LOD_{MOTHER} = 1.7$, $LOD_{FATHER} = 0$, $P_{DIFF} < 0.01$). Stronger linkage of BMI to paternally derived alleles was found at 20cM on chromosome 10 ($LOD_{IMP} = 1.1$, $LOD = 0.8$, $LOD_{MOTHER} = 0$, $LOD_{FATHER} = 1.7$, $P_{DIFF} < 0.05$). No evidence of linkage of BMI was found in areas known to contain clusters of imprinted genes on chromosomes 11p or 15. In conclusion, we have developed a variance components technique to assess parent of origin effects, and determined two areas where tentative evidence of parent specific effects in inheritance of BMI is present.

A General Framework for Comparing the Power of Mean Effects and Variance Component Linear Models for Quantitative Group Comparisons. *H.K. Tiwari¹, N.J. Schork^{1,2,3,4}*. 1) Dept Epidemiology/Biostatistic, Case Western Univ, Cleveland, OH; 2) The GENSET Corporation, 875 Prospect Street, Suite 206, La Jolla, CA; 3) Department of Biostatistics and program for population genetics, Harvard School of Public Health, Boston, MA; 4) The Jackson Laboratory, Bar Harbor, ME.

There are a number of ways one can test for the effect of certain groupings on a quantitative outcome. Standard ANOVA designs test the equality of the mean outcomes across the groups. An alternative test procedure involves determining if the amount of variation explained by a grouping is greater than 0. This alternative test procedure can be constructed as a one degree-of-freedom test of a variance component in a random effects linear model. We consider and compare the power and sample size requirements for the variance component testing approach as well as direct, ANOVA-like, tests of the equality of means across the groups using standard asymptotic theory for multivariate normal linear models. We also describe the conditions under which each test is favorable. In general, we find that ANOVA-like tests are favorable when there are few groups, but inefficient relative to the variance component tests when there are a large number of groups, due to the large number of degrees of freedom associated with testing the equality of the means. We also comment on the limitations of our comparison strategy as well as assumptions that need further consideration.

Identification of a putative recombination hotspot in the promotor of the TNFa gene. *K.C.C. Wong, D. Kwiatkowski, A.E. Richardson, W.O. Cookson, M.F. Moffatt.* WTCHG, University of Oxford, Oxford, Oxfordshire, UK.

Patterns of linkage disequilibrium (LD) with the genome may be the result of mutation, selection and drift. In addition, regions of increased recombination may contribute to the regional distribution of LD, with important implications for the LD mapping of genes influencing disease. As part of our studies of the role of tumour necrosis factor α gene (TNFa) polymorphisms in the aetiology of asthma, we have examined 10 polymorphisms in the TNFa gene and its promoter. One thousand and four subjects from 230 families of a general population sample from Busselton in Western Australia were typed, yielding a total of 10,357 genotypes. The SIMWALK2 program was used to construct haplotypes from the family data, accurately defining haplotypes spanning approximately 4.4Kb between the TNFa gene and a *Nco* I polymorphism in the first intron of the Lymphotoxin α gene. The 10 polymorphisms of TNFa are in the first 2376bp of the 4.4Kb region. Seven single recombinants within the 4.4Kb were found in 1112 meioses, when only 0.05 would have been anticipated from a typical genomic region of this extent (based on a recombination frequency of 1% / Mb) ($c^2=11122$, $p=0.00000$). Examination of pair-wise linkage disequilibrium (LD) between all markers within the region showed an area of discontinuity of LD on either side of a limited 650 bp segment sited centrally within the TNFa promoter. The region therefore contains a putative recombination hotspot, the second to be identified in the MHC region.

Frequencies of pERT87-8/Tag1 and pERT87-15/BamH1 alleles linked to the Duchenne muscular dystrophy gene in Moldavian populations. V.C. Sacara. Nat.Center of Med.Genetics , S R I C & M H C, Kishinev, Moldova.

For define pERT87-8/Tag1 and pERT87-15/BamH1 polymorphism in Moldavian population were study 15 females and 40 males (total 70 unrelative chromosomes). The RFLPs identified for pERT87-8/Tag1 are of 145bp (allele A1) and 74bp + 71bp (allele A2) and for pERT87-15/BamH1 are of 216 bp (allele F1) and 166bp + 50bp (allele F2) . These alleles were present in these ethnic group and no new allele was found. The allele frequencies calculated from the total number of chromosomes tested in population Moldova. The heterozygote numbers are counted by Hardy-Weinberg equilibrium. The allele A2 frequency (pERT87-8) in the Moldavian sample (0,815) is similar to the English population and Nigerian. The frequency of allele A1 is 0,185. The expected heterozygote number in the Moldavian population is 0,30. We also study RFLP pERT87-8/Tag1 on DMD patients (total 70 unrelative pathology chromosomes). The frequency of allele A2 is 0,428 and the frequency of allele A1 is 0,571. Since the allele A1 are found in 4 times more often on pathology chromosomes and its linked the Duchenne muscular dystrophy. The frequency of allele F1 (pERT87-15/BamH1) is 0,05 significant low and the frequency of allele F2 is 0,95. The expected heterozygote number 0.095. Polimorphism pERT87-15/BamH1 is likely to be of limited value in Moldavian population. To conclude, different populations may differ greatly in their frequencies of various RFLPs.

Fetal programming of insulin resistance: the use of twins to control for genotype. *R. Loos, R. Fagard, G. Beunen, C. Derom, R. Vlietinck.* Katholieke Universiteit Leuven, Leuven, Belgium.

Low birth weight and fetal thinness have been associated with non-insulin dependent diabetes mellitus and insulin resistance in adulthood. It has been suggested that this association may result from fetal programming in response to the intrauterine environment. An alternative explanation is that the same genetic influences alter both intrauterine growth and adult glucose tolerance. In the present study we investigated the association between birth weight and insulin resistance using twins. The use of twins allows perfect matching for gestational age, maternal height and birth order. Monozygotic twins (MZ) have identical genotypes and allow controlling for putative genetic influences. Birth records of 131 male and 145 female same-sex and 42 opposite-sex twin pairs (70 DZ, 206 MZ, 25.7 ± 4.8 years) were obtained from the East Flanders Prospective Twins Survey. Fasting plasma insulin and glucose concentrations were measured to assess insulin resistance with the homeostasis model assessment (HOMA). A multiple regression analysis was used to study the association between birth weight (BW) and insulin resistance (IR), controlling for gestational age, age, and body composition. BW explained 2% ($p=0.02$) of the variance of IR in female twins: per kg decrease in BW the IR increased with 0.25. Age and body composition explained 12%. In males, BW did not contribute significantly. We hypothesized that within pairs, the leanest (lowest BW) would have a higher IR compared to his heavier sib (highest BW) as an adult. Therefore we analyzed the intra-pair BW difference with the intra-pair IR difference at adulthood of MZ twins. An inverse relation was found between BW difference and IR difference in female twins: per kg difference in BW the leanest had a 0.43 higher IR compared to her heavier sib ($p=0.14$). In males this tendency was less apparent ($p=0.39$). These results show for insulin resistance that the fetal origin hypothesis applies to female twins, but not to male. Even in MZ pairs, sharing the same genotype, the leanest (at birth) tends to have the highest insulin resistance as an adult compared to her heavier sib (at birth).

Excess of rare polymorphisms in the genomic sequence of Fibroblast Growth Factor Receptor 3. *D.L. Lind, D.R. Cox.* Department of Genetics, Stanford University, Stanford, CA.

There are at least twelve diseases, all inherited in an autosomal dominant fashion, which are caused by recurrent spontaneous mutations in members of the Fibroblast Growth Factor Receptor (FGFR) gene family. If the high frequency of spontaneous mutations in FGFR2 and FGFR3 is caused by a high mutation rate, this might also cause a change in the amount or distribution of polymorphisms in the genomic sequence of these genes compared to genes where new mutations do not occur as frequently, such as FGFR1 or FGFR4. We sequenced PCR products covering the genomic regions for three of the FGFRs in 36 normal individuals. FGFR2 and FGFR3 covered 48 and 14 kb, respectively, and are used as examples of regions with a potentially high mutation rate. FGFR4 covered 12 kb, and is used as an example of a region with a potentially lower mutation rate as no disease-causing mutations have yet been identified in this gene. We found no significant difference in the overall amount of sequence variation in these three genes, though we did find a difference in how the variation is distributed. Using Fu and Li's D^* test, for FGFR2 and FGFR4 the results were slightly negative, though not significantly different from neutral expectations. For FGFR3, D^* is -2.42 ($p < 0.025$). A negative D^* value indicates an excess of rare polymorphisms relative to the overall amount of variation, and may be the result of population expansion, population stratification, or a selective sweep. Population expansion or stratification is unlikely to be able to account for such a large deviation from neutral, though may account for some of the difference. This would suggest a selective sweep has taken place in FGFR3, removing some of the common polymorphisms.

Mitochondrial DNA diversity in East European populations. *O.V. Belyaeva¹, S.P. Markina¹, M.A. Bermisheva², N.A. Bebyakova³, A.I. Mikulich⁵, L.A. Livshitz⁴, E.K. Khusnutdinova², S.A. Limborska¹*. 1) Human Molecular Genetics, IMG RAS, Moscow, Russia; 2) Inst. of Biochemistry and Genetics, Ufa, Russia; 3) Arkhangelsk State Medical Academy, Arkhangelsk, Russia; 4) Inst. of Molec. Biology and Genetics, Kiev, Ukraine; 5) Inst. of Ethnography and Anthropology, Minsk, Republic of Belarus.

Mitochondrial DNA diversity widely employed in population and evolutionary studies is investigated insufficiently among East European peoples. We have performed the mtDNA sequence analysis in a set of population from Russia and neighboring countries underrepresented in worldwide mitochondrial polymorphism studies. The first hypervariable segment of the mitochondrial control region was sequenced in representative samples of native Slavic population of Belarus, Ukraine and Russia, including very peculiar isolated rural populations. Frequencies of main haplogroups were determined based on phylogenetically informative positions of HVSI. The median network constructed for samples studied revealed branches corresponding to main haplogroups determined from these positions. The comparison of haplogroup frequencies, as well as intrapopulation diversity characteristics obtained to mitochondrial DNA data described by others for previously studied European and Asian samples revealed that variations among our samples are within European mtDNA variations in total. Nevertheless the comparison of different East European populations to each other revealed the complicated structure of Russian gene pool. The distribution of mtDNA variations between different Russian samples reflects old migrations proposed by historical data.

Genome-wide distribution of linkage disequilibrium in the population of Palau and its implications for gene flow in Remote Oceania. *W.F. Byerley¹, K. Roeder², K. Otto³, S. Tiobech³, B. Devlin⁴.* 1) Bldg 837, Rm 2119, ZC 4260, Univ California, Irvine, Irvine, CA; 2) Department of Statistics, Carnegie Mellon University, Pittsburgh, PA; 3) Palau National Hospital, Palau; 4) Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA.

Linkage disequilibrium (LD) between alleles on the same human chromosome results from various evolutionary processes and is thus telling about the history of populations. Recently, LD has garnered substantial interest for its value to map and fine-map disease genes. We examine the distribution of LD between Short Tandem Repeat (STR) alleles on autosomes and between STR alleles on sex chromosomes in the Remote Oceanic population of Palau to evaluate two related issues: (1) Are the data consistent with a recent hypothesis about the origins of genetic variation in Palau, specifically that the population experienced extensive male-biased gene flow following initial settlement (Lum et al., *Am J Hum Genet* 63:613-624); and (2) Could the Palauan population be useful for LD mapping of disease genes due to its population history? Consistent with evolutionary theory based on effective population size, LD between X-linked alleles is stochastically greater than LD between autosomal alleles; however, small but detectable LD occurs for autosomal markers separated by substantial distances. By contrast, while Y-linked alleles experience only one-third the effective population size of X-linked alleles, their mean value for pairwise LD is slightly smaller than X-linked alleles. For a small population known to experience at least two extreme bottlenecks, Y haplotypes exhibit remarkable diversity (0.96, based on 6 STRs) and fall into six distinct clusters. We argue these data are consistent with waves of male-biased gene flow. Theoretical analyses, combined with the empirical LD analyses, also show that Palau could be a promising population for LD mapping of disease genes.

Estimating the rate of decay of linkage disequilibrium with distance. *L. Frisse¹, A. Bartoszewicz¹, A. Pluzhnikov¹, R. Hudson², A. Di Rienzo¹.* 1) Human Genetics, University of Chicago, Chicago, IL; 2) Ecology & Evolution, University of Chicago, Chicago, IL.

The rate at which linkage disequilibrium (LD) decays with physical distance in human populations is of great interest for designing disease association studies. In addition to the rate of mutation and recombination, LD is affected by two main evolutionary forces: population history and natural selection, which operate on different scales. Natural selection affects LD locally, while population history typically has genome-wide effects. In order to characterize how LD was shaped by population history in human populations, we have developed an efficient scheme for surveying LD in neutrally evolving genomic regions. Several independent regions of 10 kb are amplified and segments of 1 kb at each end of these regions are sequenced in population samples from three major ethnic groups. The haplotype phase is subsequently determined by cloning the 10 kb fragment of each multiply heterozygous individual. This scheme for data collection allows us to estimate the extent of LD over the entire frequency spectrum, which is informative for the reconstruction of population histories. In particular, we can survey LD within each 1 kb segment as well as between segments over a 10 kb distance. The preliminary results of this survey show that LD between sites separated by distances of up to 1 kb is not complete while it shows substantial, but incomplete decay between sites separated by distance of up to 10 kb. Furthermore, LD appears to decay at a different rate in different ethnic groups.

Skin pigmentation and individual admixture estimates. *H.L. Norton¹, C.L. Pfaff¹, E.J. Parra¹, N. Sylvester², D. Parrish-Gause², R.A. Kittles², M.D. Shriver¹*. 1) Pennsylvania State University, University Park, PA; 2) Howard University Cancer Center, Washington, D.C.

Variation in human pigmentation has been studied objectively using reflectance spectroscopy for over 50 years. The identification of the genes responsible for pigmentary variation among human populations is the first step in understanding the molecular and evolutionary history of human pigmentation. African-American populations provide a valuable opportunity to investigate the genetics of pigmentation due to their unique demographic history. DNA samples were collected from 162 African-American individuals from Washington D.C. and genotyped for 11 population-associated alleles (PAAs). Skin reflectance measurements were taken from the subjects using the DermaSpectrometer instrument, which separates the reflectance due to melanin absorbance from that of hemoglobin and summarizes this value as the Melanin (M) index. Individual admixture proportions were calculated from the PAA multilocus genotypes using maximum likelihood. Regression analysis showed a significant correlation between the estimate of percent of African ancestry and M index ($R^2 = 0.15$, $p < 0.001$). Individuals showing lower values of the M index (i.e. lighter skin color) showed lower percentages of African ancestry. One major source of variation (as seen in the low R^2) may be the small number of PAAs available, resulting in imprecise individual admixture estimates. We then tested for association between any PAA and M index with individual admixture as a covariate. Two of the PAAs that are also pigmentation candidate genes, OCA2 and GC (KIT), were significantly associated with higher M indices even after individual admixture was taken into account. These results demonstrate that phenotypes that are divergent in parental populations may reflect the proportion of admixture in individuals of admixed populations. We also provide an example of a way to use the individual admixture estimate to identify the genes underlying the phenotype. The important consequences for research on complex diseases in African-American and Hispanic populations are also discussed. This work was supported by a grant from NIH/NHGRI (HG02154).

Q318X is the Common Mutation of CYP21 gene in Tunisian Population. *M. Kharrat¹, R. Mrad^{1,2}, N. Smaoui^{1,2}, F. Maazoul², A. Masmoudi², L. Ben jema^{1,2}, H. Chaabouni^{1,2}.* 1) Laboratory of Human Genetics. , Medical School Tunis, Tunis, Tunisia; 2) Department of Hereditary Diseases .Charles Nicolle hospital. Tunis,Tunisia.

Congenital adrenal hyperplasia is an autosomal recessive disease with heterogeneous phenotype. The most common form is due to 21-hydroxylase deficiency (21-OHD) resulting from lesions in the CYP21 gene. Up to date more than 20 different mutations have been reported in CYP21 gene, word distributed. Mutations analysis have been investigated in 50 unrelated Tunisian families. All affected members have classic phenotype with severe salt wasting syndrome and impaired cortisol and aldosterone synthesis. Molecular study of 100 chromosomes shows that in 38 % Q318X mutation (GlyStop at 318) that abolishes a site for PstI cleavage is present. Comparing our results with those observed in Caucasian population where the incidence is about 1 to 10 %, leads us to conclude that the frequency of Q318X seems very high in Tunisian population. According to high incidence of consanguinity in affected families (70 %) it could be possible to explain Q318X mutation frequency by a founder effect in our region.

Allelic homoplasy of STR due to variable allelic flanking sequences: SNPs in STR. *W. Wang, A.H. Bittles.* Centre for Human Genetics, Edith Cowan University, Perth, Australia.

Single tandem repeats (STRs) and single nucleotide polymorphisms (SNPs) are widely used as markers in human genome studies. We have characterized a highly polymorphic STR locus (D20S85) with (AAAG)_n repeats, by a combination of direct DNA sequencing and single-strand confirmation polymorphism (SSCP) analysis. Eight STR alleles were identified on denaturing gels, and SSCP gels (6% acrylamide/bisacrylamide containing 5% glycerol, 400x200x0.35mm) were then used to demonstrate homoplasy of the locus on the basis of variable allelic flanking sequences. This was confirmed by direct sequencing of the alleles using a Taq Dye Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Four transitions, two of G to A and two of A to G, occur in the 5' flanking region of the locus at positions 14, 22, 24 and 26, which effectively subdivide the STR alleles into two groups. The results indicate that the mutational processes which generated the polymorphisms involved both simple changes in the number of AAAG repeats, and single nucleotide mutations in the region flanking the repeat, i.e. SNP changes. Using direct analysis of SSCP gels, the frequencies of the two sets of sequence polymorphisms were estimated to be 0.431 and 0.569 respectively. The two families of markers exist at the same locus and give rise to STR homoplasy involving allele size and composition. Thus the findings have potential application in the avoidance of false linkage and association. More importantly, a locus of this nature, with separate STR and SNP evolutionary histories and resulting from different mutation processes, should have wide application in studies of selection, drift, migration and inbreeding.

Intraethnic genetic differentiation of Evenks by mtDNA polymorphism. *T.P. Muravyova*^{1,2}, *M.V. Goloubenko*¹, *V.P. Puzyrev*^{1,4}, *V.B. Salioukov*^{1,4}, *E.P. Bychina*^{1,3}. 1) Institute of Medical Genetics, Tomsk, Russia; 2) Institute of Pediatrics, Chita, Russia; 3) Tomsk State University, Tomsk, Russia; 4) Siberian Medical University, Tomsk, Russia.

Mitochondrial DNA polymorphism in the sample of Evenks living in Chita region of Russia (three settlements, N=85) has been investigated by restriction and length polymorphisms (HaeIII663, DdeI 10394, AluI 5176, 10397, 13262, V region length polymorphism) to determine the spreading of the A, B, C, D mtDNA haplogroups in Evenk population. The sample harbors haplogroups A, C and D with overall frequency as 76.18% with the prevalence of haplogroups C (35.71%) and D (34.52%), and with the frequency of haplogroup A about 6%. Some other lineages have been revealed. Evenks living in Chita region lack the deletion in V region and, consequently, haplogroup B. The investigated sample has been compared with the Evenks living in Krasnoyarsk region (N=51), studied by A. Torroni et al. (1993). In Krasnoyarsk sample, most of mtDNAs belong to the haplogroup C (84.31%), with the frequencies of A and D about 10% and 4% respectively. So, although these two populations have similar spectrum of main haplotypes (A, C, and D), they differ significantly by their frequencies. Such intraethnic differentiation may be the result of the population history of Evenks, which are not numerous but at the same time live separately on the large territory of Siberia, so genetic drift may significantly influence the mtDNA polymorphism.

Evolution of Y-chromosome haplotypes in populations of North Eurasia. *V.A. Stepanov^{1,2}, V.P. Puzyrev¹*. 1) Inst for Medical Genetics, Tomsk, Russia; 2) Tomsk State University, Tomsk, Russia.

Distribution of Y-chromosome haplotypes in 18 native populations of Siberia and Middle Asia was investigated in order to reconstruct the evolution of paternal lineages in North Eurasia. Compound Y-chromosome haplotypes were constructed using four biallelic loci (SRY3225, YAP, DYF155S2 and Tat) and seven microsatellites (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392 and DYS393). Automated genotyping of STR loci was performed with HEX-, TET- or FAM-labeled primers with the ABIPrism310 genetic analyzer and Genescan software. High level of gene diversity ($H=1.00$) and substantial degree of genetic differentiation of male-specific gene pool ($F_{st} = 0.212$, $P=0.000$) in modern North Eurasian populations were found. Haplotypic lineages and their evolutionary relationships were reconstructed. It was shown that most haplotypes in modern North Eurasian population have common ancestry traced back to the Upper Paleolithic period (about 15000 years ago). Using the molecular variance of microsatellites within the biallelic haplogroups the age and origin of ancestral haplotypes for DYF155S2 - (6900 years) and TatC (4200 years) lineages, specific for population of North Eurasia were estimated. Phylogenetic analysis of genetic relationships between populations, performed with the Y-chromosome haplotypes, reveals the close affinity of Y-chromosome gene pool in Altaic populations of Middle Asia and Indo-European populations on the one hand and clear clustering of Turkic-speaking Siberian ethnic groups on the other hand.

TSER Length Polymorphism in Chinese population: Allele Frequency and mechanism of evolution of the Novel Alleles. *H-R. Luo, Y-P. Zhang.* Lab Cellular & Molecular Evolution, Kunming Institute of Zoology, Kunming, Yunnan 650223, China.

Thymidylate synthase (TS) is an essential enzyme that must be expressed in all proliferating cells and a target enzyme for cancer chemotherapy drugs such as 5-fluorouracil (5-FU). The tandemly repeated 28-bp segment in the 5'-terminal regulatory region of the human thymidylate synthase (TSER), which have been reported to be polymorphic in different populations, was surveyed in 668 Chinese from 9 Han geographic groups and 8 ethnic populations, 36 individuals representing a three-generation pedigree and three apes. Amplified fragments were analyzed by electrophoresis on 4% agarose gel. In addition to the reported double and triple repeats of the 28-bp segment in TSER, we also detected 5 repeats in this region. All the three alleles of the repeat type (2,3 and 5) were further confirmed by direct sequencing. Based on the pedigree analysis, these three alleles were found to be inherited in accordance with Mendel's laws. By direct comparison of the repeat types among apes and human, the length polymorphism in TSER could be explained by slipped-strand mispairing (SSM) during DNA replication. The frequencies of the TSER allele 2 and 3 were 18.82% and 81% in unrelated whole Chinese samples. The highest frequency of allele 3 was found in Wa population (95%) from Yunnan province, while the lowest was presented in Kazak (62%) and Uygur (63%) from Xinjiang, with values that are similar to the reported Caucasian's (60%). The remained 14 ethnic populations had a considerably high frequency with a range from 72% to 90%. Furthermore, the distribution of the TSER polymorphism in world populations might be helpful to understand the relationships of the racial and ethnic groups.

Sampling properties of estimators of nucleotide diversity at discovered SNP sites. *A. Renwick¹, P. Bonnen², D. Triikka², D. Nelson², R. Chakraborty³, M. Kimmel¹.* 1) Department of Statistics, Rice University, Houston, TX; 2) Dept. Human and Molecular Genetics, Baylor College of Medicine, Houston, TX; 3) Human Genetics Center, Univ. Texas School of Public Health, Houston, TX.

SNP sites are discovered generally by sequencing regions of the genome in a limited number of individuals. This may leave SNP sites present in the region, but containing rare mutant nucleotides, undetected. Consequently, estimates of nucleotide diversity obtained from assays of detected SNP sites are biased. In this research we present a statistical model of the SNP discovery process, which in turn is used to evaluate the extent of this bias. Under various models of gene frequency distributions at the SNP sites, we show that nucleotide diversity is always underestimated. However, the extent of bias rarely exceeds 10%. This same effect leads to an underestimation of the number of segregating sites as well. Applications of this theory to new SNP data at ATM, BLM, RQL and WRN gene regions as well as to the literature data at the LPL gene region indicate that in spite of such ascertainment biases, the observed differences of nucleotide diversity across these gene regions are real. This suggests heterogeneity of rates of nucleotide substitution rates across the genome. (Research supported by US Public Health Service Research grant GM 41399 and CA 75432 from the National Institutes of Health and a NSF graduate fellowship to AR).

Minisatellite DNA diversity in East Slavs. *D.A. Verbenko¹, O.P. Balanovsky¹, T.V. Pogoda¹, A.I. Mikulich², L.A. Livshits³, L.V. Bets⁴, N.A. Bebyakova⁵, S.A. Limborska¹*. 1) Institute of Molecular Genetics, Moscow, Russia; 2) Institute of Arts, Ethnography and Folklore, Minsk, Byelorussia; 3) Institute of Molecular Biology and Genetics, Kiev, Ukraine; 4) Moscow State University, Moscow, Russia; 5) Arkhangelsk State Medical Academy, Russia.

Genetic diversity in native peoples from Russia, Byelorussia and Ukraine has been analyzed. The polymorphism of hypervariable DNA markers, namely minisatellite loci D1S80 (pMCT118) and 3'ApoB was examined. More than 1000 unrelated individuals from 18 Slav population samples were studied.

Allele typing was performed using the PCR and subsequent electrophoresis followed by silver staining. We detected 25 alleles of the D1S80 locus (from 16 to 41 tandem repeats) and 23 alleles of the 3'ApoB locus (from 25 to 53 tandem repeats). Genotype frequencies for these loci in all populations met Hardy-Weinberg expectations. Observed allele distributions were similar to other Caucasoid populations ones.

Genetic distance analysis demonstrated that genetic diversity of the 3'ApoB locus was greater in comparison to the D1S80 locus. Genetic trees were constructed and multidimensional scaling of allele distributions with allocation populations in multidimensional space was made. These methods indicate certain differentiation between ethnic groups of East Slavs. In spite of great heterozygosity (around 0,8) and high degree of polymorphism in minisatellite loci Nei's G_{ST} value is less in four times in contrast to classical immunobiochemical markers. The method of computer genegeography with reliability theory was used to prepare maps of geographic variability for all alleles. East-West gradient of variability was traced on the first principal component map for the D1S80 locus. We also investigated Yakut population belongs to Mongoloid group. Allele distribution in Yakut statistically differed from East Slavs but simulate with other Asian populations.

Short tandem repeat-based Y-chromosome haplotype data reveals a high level of admixture in the migrant population, the Siddis, with local Indian populations. *L. Singh*¹, *G.V. Ramana*², *N. Wang*², *R. Chakraborty*². 1) Director, Ctr Cellular & Molec Biol, Hyderabad, Andhra Pradesh, India; 2) Human Genetics Center, Univ. Texas School of Public Health, Houston, TX.

Earlier studies on haplotype distributions based on short tandem repeat (STR) loci in the Indian subcontinent reflected negligible male gene flow across populations. To investigate whether it holds for the migrant population, the Siddis (a population of African descent), we analyzed five STR loci (DYS19, DYS389, DYS390, DYS391, and DYS393) in 94 males belonging to two caste populations (Brahmins and Kammas) and the migrant Siddis of Southern India. We observed 83 different haplotypes in our sample. In other words, like the data of the literature, we also observed low levels of haplotype sharing between populations. Utility of these markers for differentiating populations of the Indian subcontinent is studied through a phylogenetic analysis of haplotype distributions in our samples together with data from 12 other populations from India, Pakistan, and Ethiopia. With haplotypic distinctions measured in terms of repeat size differences at these five STR loci, we observe that the populations cluster by their geographic location, rather than by their caste affiliation. This is consistent with the sociological theory of caste origin in India. Additionally, the Siddis clustered with the two South Indian caste groups, rather than being closer to the Ethiopian population, from where they are presumed to have migrated. Coalescence theory-based estimation revealed that the Siddis, in their male gene pool, has approximately 60% genes of local origin, a value somewhat smaller than the 72% admixture estimate, based on nuclear blood group and protein polymorphism data. From these observations, we conclude that restricted male gene flow does not appear to be true in this migrant population, and the presumed theory that only male migrants arrived from Africa to establish the community of the Siddis in India does not have a genetic support. (Research supported by US Public Health Service Research grant GM 41399 from the National Institutes of Health).

Genetic estimation of biogeographical ancestry. *C.L. Pfaff, E.J. Parra, M.D. Shriver.* Pennsylvania State University, University Park, PA.

Ethnicity is comprised of both biological and cultural components. Biogeographical ancestry (BGA) refers to the component of ethnicity that is biologically determined and can be estimated using genetic markers that have distinctive allele frequencies for the populations in question (referred to as population-associated alleles - PAAs). We have developed a method that uses a maximum likelihood (ML) approach to estimate the primary population source(s) of an unknown DNA sample. Once the potential source populations have been narrowed to the two or three populations with the highest log likelihood ratios (LLR), individual admixture proportions are estimated for the multilocus genotype observed in order to characterize the proportional ancestry of the sample.

We have explored the potential of this method by examining the multilocus genotypes of African, European, and African-American DNA samples using a panel of 10 PAAs that have high allele frequency differences between Africans and Europeans. In each of the 906 African and European samples BGA was correctly estimated using a maximum likelihood approach. In 863 cases the LLR for the estimation was > 3 (avg. LLR = 4.7), indicating a strong confidence in the estimation of ancestry. However, as expected, the ML estimate is less precise for African-American samples. In these cases the inaccurate and low-confidence estimates tend to be for individuals with relatively high admixture proportions, making population distinctions more difficult. A second source of inaccuracy is the relatively low number of informative markers currently available. In order to examine this cause, we simulated 2000 individuals with multilocus genotypes at 20 loci. Of these, ancestry estimation was correct in every case, and only 2 individuals had LLR < 3 . While the utility of this method is currently limited by the restricted number of PAAs available for various populations, it is clear that as larger numbers of ancestry-informative markers become available, estimation of BGA may become a powerful tool for the elucidation of an individual's genetic and population history, as well as the identification of unknown samples in forensic cases.

Pattern and dynamics of admixture in US African-American populations: a global perspective. *E.J. Parra¹, C.L. Pfaff¹, K. Hiester¹, D. Sosnoski¹, Y. Jien¹, F. Gulden¹, P. McKeigue², M.D. Shriver¹*. 1) Dept Anthropology, Penn State Univ, University Park, PA; 2) Dept Epidemiology and Population Health, London School of Hygiene & Tropical Medicine. London, UK.

Initial attempts to characterize admixture in African Americans using genetic markers date back to the 50s. Since then, many studies have been devoted to this subject, yet no single survey has attempted a comprehensive study of admixture in African Americans from many different geographic areas, using a select panel of ancestry informative markers. We have been studying admixture in 16 geographically diverse African-American populations using 10 autosomal genetic markers showing large frequency differences between the relevant parental populations, as well as informative mtDNA and Y-specific polymorphisms. We observe that the European contribution to African Americans ranges from 3.5 to 25 per cent in these populations. The geographic pattern of admixture is complex, but can be interpreted in light of historical and demographic information. We consistently find a higher genetic contribution from European males than from European females, indicating sex-biased gene flow. In some African-American populations, we see evidence of a Native American genetic contribution, but globally this contribution is low (less than 2 per cent). Using computer simulations, we have compared the effect of two admixture models (continuous gene flow and hybrid isolation), in terms of the associations created between linked and unlinked markers. In our samples, we observe significant associations between linked markers, but also between unlinked markers in many of the pairwise comparisons. These results are consistent with the simulations of the continuous gene flow model, although other factors, such as assortative mating, could also explain the observed results. We have seen, both in our population samples and in the computer simulations, that irrespective of the admixture model, the linkage disequilibrium introduced by the admixture process can be used for mapping genes. This work provides further insights regarding admixture and the applicability of admixture mapping in African American populations.

(AC)_n dinucleotide repeat polymorphism in 5B-globin gene in native and Mestizo Mexican population., F.

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Repeated sequences are dispersed along the human genome. These sequences are useful as markers in diagnostic of inherited diseases, forensic medicine and in tracking the origin and evolution of human populations. (AC)_n repeated element is the most frequent in human genome. In the present paper, (AC)_n repeated element located in the 5' flanking region of the globin gene was studied by SSCP in four ethnic Mexican groups (Mixteca, Nahuatl, Otom, Purpecha) and in a Mestizo population. We observed three alleles called A [(AC)₁₆], B [(AC)₁₄] and C [(AC)₁₈] with a frequency between 68.2% and 86.9% for first case; between 13.1% and 18.2% for second one and finally 6.7% and 13.6%, in the last one. The allele C was present only in Purpecha and Mestizo groups, suggesting evidence about the different origin of Purpecha group. The absence of this allele in the other ethnic groups studied also suggests that there is no genetic admixture with the Purpecha and that this is a relative isolated population. The (AC)_n repeat polymorphism in the globin gene has not been previously studied in Amerindian populations.

Evidence for marked genetic differentiation within and among eastern rural Chinese population. *M.T. Seielstad¹, X. Xu¹, M. Wang¹, B. Wang¹, J. Rogus¹, D. Wu⁴, X. Xu¹, N.J. Schork^{2,3}*. 1) Program Population Genetics, Harvard Sch Public Health, Boston, MA; 2) Dept of Epidemiology & Biostatistics, Case Western Reserve University, Cleveland, OH; 3) Genset Corp., LaJolla, CA; 4) Anhui Medical University, Hefei, China.

Considerable genetic variation is known to exist among and between individual humans and populations. The degree to which this variation correlates with historical views of the origin and relationships of racial and ethnic categories is unclear and controversial. For example, it is generally acknowledged that there is often more genetic variation within many racial groups than there is between or across racial groups. The degree and causes of this variation are the subject of a great deal of current research. In this paper, we consider evidence for genetic differentiation in eastern rural Chinese communities gathered from three independent studies. We find striking evidence for genetic differentiation, especially with respect to Y chromosome variation. We argue that this differentiation likely reflects both social practices in rural China as well purely geographical and mobility factors. Our studies document the utility of assessing genetic variation within groups thought to be fairly homogeneous to settle questions about the degree and causes of genetic and phenotypic variation in the population at large,.

Population diversity of a 45 bp VNTR associated with Usher Syndrome Type IC and exclusion of a positional candidate gene. *S. Savas*¹, *B. Frischhertz*², *M. DeAngelis*¹, *Z. Den*¹, *J. Doucet*³, *M. Pelias*¹, *C. Petit*⁴, *P. Deininger*², *M. Batzer*¹, *B. Keats*¹. 1) LSU Health Sciences Center, New Orleans, LA; 2) Tulane Cancer Ctr. New Orleans, LA; 3) Nicholls State Univ. Thibodaux, LA; 4) Institut Pasteur, Paris, France.

Type IC Usher syndrome is an autosomal recessive disorder characterized by deafness, absent vestibular function, and progressive retinal degeneration. The USH1C gene was localized to chromosome 11p in Acadian families. Lebanese families mapping to this region have also been reported. Additionally, DFNB18, an autosomal recessive deafness locus, was mapped to the USH1C region. The DelGEF gene, isolated from that region, was suggested as a positional candidate for USH1C and DFNB18. We excluded DelGEF as the USH1C gene by direct sequencing. Recent evidence suggests that a gene encoding a PDZ domain containing protein may be the USH1C gene. Sequence analysis of DNA samples from Acadian USH1C patients did not detect a mutation in the coding region, but a nine times tandemly-repeated 45 bp VNTR in intron 5 was homozygous in all Acadian patients. In a control population, 2, 3, 6, and less frequently 4 and 8 repeats were found. To determine the distribution of this VNTR in different populations (Asian, African-American, Hispanic, African, European, Acadian) we screened 390 unrelated individuals. In one heterozygous Hispanic sample, a fragment larger than 9-repeats was amplified. Also, a fragment size corresponding to the 9-repeat Acadian allele was detected in a heterozygous Hispanic sample. In one Hispanic and one African heterozygous sample, we detected the presence of a single repeat allele. Two other previously unreported alleles (5 and 7 repeats) have been detected with frequencies of 0.25% and 0.5%. The most frequent alleles in the combined populations were 2, 3, and 6 repeats (46.3%, 25.6%, and 23.9%, respectively). The repeat region could also be amplified from several primates and rodents suggesting evolutionary conservation.

Analysis of the *UGT1A1* promoter polymorphism in a population of patients with hyperbilirubinemia and suspected Gilbert syndrome. S. Das, M. Song, D. Shamoun, J.L. Jensen. Department of Human Genetics, University of Chicago, Chicago, IL.

Gilbert syndrome is a benign form of unconjugated hyperbilirubinemia. It occurs at a very high frequency with an estimated incidence of 2-12% in the general population. A polymorphism in the promoter of the UDP glucuronosyl transferase 1 (*UGT1A1*) gene, in which 7 instead of 6 TA repeats occur, has been identified, and homozygosity for the '7' allele has been associated with Gilbert syndrome. Ninety one patients with hyperbilirubinemia and/or jaundice and a suspicion of Gilbert syndrome were referred to our DNA diagnostic laboratory between June 1998 and June 2000 and were analyzed for the *UGT1A1* promoter polymorphism. Patients were primarily of caucasian origin and were made up of 67 males and 24 females with 7 patients being less than 9 months of age. DNA samples were fluorescently amplified for the *UGT1A1* promoter polymorphism and sized on a polyacrylamide gel. Of the 91 patients, 65 had a 7/7 genotype, 15 had a 6/7 genotype and 11 had a 6/6 genotype. The 7/7 genotype frequency in this population was 0.7 while the frequency of the '7' allele was 0.8. The frequency of the '7' allele in this group of patients is over twice that observed in the general caucasian population which has been calculated to be 0.38 in previous studies. This association is highly significant ($p < 0.0001$) and these results further support a strong association between the '7' allele and Gilbert syndrome in the caucasian population. The frequency of the '7' allele in those patients less than 9 months was 0.57 indicating that factors other than Gilbert syndrome are more likely to account for the hyperbilirubinemia in this group, however a larger number of patients would need to be studied to say this definitively. Genotyping of the *UGT1A1* promoter polymorphism provides a useful molecular test for Gilbert syndrome. Such a test is clinically useful as a positive result provides a definite diagnosis for Gilbert syndrome, where diagnosis has generally been based on exclusion. Patients are therefore relieved of further anxiety and any lingering doubts of a more serious disorder.

Heritability estimates of intelligence in twins: effect of chorion type. *C. Derom¹, S. Van Gestel¹, N. Jacobs¹, E. Thiery^{2,3}, R. Derom³, P. Vernon⁴, R. Vlietinck¹*. 1) Catholic University Leuven, Belgium; 2) University Gent, Belgium; 3) Association for Scientific Research in Multiple Births, Belgium; 4) University Western Ontario, Canada.

Twins are widely used to estimate the genetic and environmental components of complex traits in the general population. The classical twin study is based on the assumption that monozygotic twins (MZ) are genetically identical, whereas dizygotic twins (DZ) share on average half of their genes. The different prenatal environment of MZ monochorionic twins (MZ-MC) as compared to the MZ dichorionic (MZ-DC) twins is seldom taken into account. However, MC placentation allows the exchange of blood, hormones, enzymes, etc during prenatal life. As predicted by the fetal-origin hypothesis, MZ-MC twins might therefore show a higher concordance rate for some phenotypes in later life, due to a more similar prenatal environment. This study investigates the basic assumption of homogeneity of MZ twins on the heritability estimates of IQ. We determined the IQ of 451 same-sexed twin pairs of known zygosity and chorion type with the Wechsler Intelligence Scale for Children-Revised (WISC-R). The twins (181 DZ, 95 MZ-DC, 175 MZ-MC, aged 8-14 yrs) were selected at random from the East Flanders Prospective Twins Survey. Zygosity was determined by placental examination, blood groups and DNA fingerprints, reaching a probability of at least 0.99. We adapted the classical genetic model fitting by including a chorion type factor. High heritability estimates were found for almost all subscales and IQ-scores. The heritability of total IQ was 83%, when MZ twins were considered as a homogeneous group. A significant effect of chorion type was found on the subscales Arithmetic and Vocabulary: as hypothesized the MZ-MC twins resembled each other more than the MZ-DC twins. The effect accounts for respectively 14% and 10% of the total variance. Although the effect of chorion type does not play in all measures and although the contribution is moderate, it constitutes a possible confounder and urges twin researchers to take chorion type into account. As far as the heritability of total IQ is concerned, no chorion effect was found.

Frequency of the hemochromatosis associated mutations C282Y and H63D in Argentina. *F.F. Fay¹, M.E. Campodonico¹, M. Taborda¹, G. Revelant¹, H. Tanno², M. Lopez³, O.H. Fay⁴.* 1) Dept Molecular Biol, CIBIC, Rosario, Argentina; 2) Hospital Centenario, Rosario; 3) CAC-ALAC, Corrientes; 4) CTSP-UNR, Rosario.

Background: In north-European populations Hereditary Hemochromatosis (HH) is caused in 90-100% of the cases, by two common mutations of the HFE gene, and affects 1 in 300 individuals. However, lower mutation prevalences were found in different ethnic groups. Objectives: To determine the prevalence of the HFE gene HH associated mutations (C282Y and H63D) in the general Argentine population. Methods: Three hundred and fifty unrelated healthy subjects were studied for the C282Y and H63D mutations. DNA was extracted from blood EDTA samples. Mutations detection was performed using a multiplex - ARMS test. Genotypic results were correlated with serum iron and transferrin saturation (TS) levels. A group of 21 clinically diagnosed HH patients, was also studied for those mutations. Results: We found that 13 out of 350 subjects (3.7%) were heterozygous for the C282Y mutation; no homozygotes were found; 97 out of 350 subjects (27.7%) were H63D heterozygous and eleven were homozygous (3.1%). Only three subjects (0.9%) were heterozygous for both mutations (compound heterozygotes). The observed frequencies are consistent with those predicted by the Hardy-Weimberg equation. Genotypic-phenotypic correlation showed that the H63D homozygotes have a significantly higher TS mean value compared with wild type subjects ($p=0.007$). In the HH group, 7 out of 21 (33.3%) were C282Y homozygotes, 5 out of 21 (23.8%) were H63D homozygotes, and no compound heterozygotes were found. Conclusions: We found a lower prevalence of the C282Y mutation, compared with that observed in other caucasian populations. According to our observations, the carrier frequency for C282Y is 1 in 27 with an expected homozygous frequency of 1 in 2800 in the general population. Our findings are in accordance with published data, taking into consideration that our population is a mixture of Spanish, Italians, a lower degree of Portuguese, native Indians and other minor ethnic groups. Regarding the H63D, our findings suggest that the H63D mutation alone, could have a role in iron overload development in our population.

Assessing risk factors of human complex diseases by Akaike and Bayesian information criteria (AIC and BIC).

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The complex etiology of many human diseases requires a new framework for data analysis in epidemiology and genetic epidemiology (as well as pedigree linkage analysis). The traditional frameworks of hypothesis testing and maximum likelihood have their limitations. In hypothesis testing, only a simple enough question can be answered with certainty; but for complex diseases, the question posed by a usually unrealistic null hypothesis can be far from relevant. In the likelihood framework, parameter estimation is meaningful only if the model used is correct; but one typically does not know which model describes the relationship between risk factors and a complex disease. We propose the use of Akaike information criterion (AIC) and its close relative, Bayesian information criterion (BIC), in data analysis of complex diseases. AIC is solidly founded in information theory, and is a natural extension of both the maximum likelihood and likelihood ratio test frameworks. We have applied AIC/BIC to assess risk factors in three data sets: child asthma, alcoholism, and limb-girdle muscular dystrophy. We are able to address the following issues: measuring the amount of information (non-randomness) in the data set, comparing between single-factor and all-factor analysis, excluding irrelevant factors, selecting important factors, determining (roughly) the number of factors involved, selecting between competing models such as logistic regression and neural networks, and delineating range of models by using BIC to select the under-fitting model. All these issues can be studied within a single consistent framework using a single measurement (AIC or BIC).

Myotonia congenita in Northern Norway shows high frequency and a characteristic spectrum of *CLCN1*

mutations. C. Sun¹, M. Van Ghelue¹, T. Torbergesen², E.P. Hoffman³, L. Tranebjærg¹. 1) Dept. of Medical Genetics, University Hospital of Tromsø, Tromsø, Norway; 2) Dept. of Neurology, University Hospital of Tromsø, Tromsø, Norway; 3) Research Center for Genetic Medicine, Children's National Medical Center, Washington DC, USA.

Myotonia congenita is a genetic muscle disorder characterized by hyperexcitability of the skeletal muscle membrane. Mutations in the muscle chloride channel gene, *CLCN1*, lead to either autosomal dominant (Thomsen's) or autosomal recessive (Becker's) myotonia. We have investigated 18 families from Northern Norway and Northern Sweden in order to characterize the spectrum of *CLCN1* mutations and detect possible genetic heterogeneity. Direct sequencing of all 23 exons was undertaken in 18 probands, and 53 relatives were studied with respect to the relevant mutations detected. We found a high prevalence of 9.4:100,000 in Northern Norway, similar to Northern Finnish figures. Eight *CLCN1* mutations and three polymorphisms (T87T, D718D, P727L) were identified. Three mutations (F287S, A331T, 2284+5C>T) were novel. Three previously reported mutations, A531V, F413C, and R894X, accounted for 42% of the identified mutations in all patients. The majority of our cases was compound heterozygotes of which the combination A531V/R894X dominated. The same three mutations comprised the entire *CLCN1* mutational spectrum in Northern Finland where F413C was predominating. A reliable genotype-phenotype correlation could only be established for patients with A531V/R894X. Variable penetrance was found in patients being heterozygous for either. The population frequencies of A531V and R894X were 0.3% and 0.87%, respectively. Our findings support that myotonia congenita is significantly more prevalent in Northern Scandinavia than elsewhere. Possible explanations include fairly high population frequencies of particular *CLCN1* mutations, selective advantages of heterozygotes, cold climate and yet unidentified genetic factors. Future studies aim at expression studies of identified missense mutations and clarifying the apparent genetic heterogeneity illustrated by the fact that 2/18 probands had no *CLCN1* mutations detected.

Beta-globin gene cluster haplotype frequencies in Colombia suggest different origins for Afro-Caribbean populations. *M.C Mondragon*¹, *F. Cuellar-Ambrosi*¹, *G. Bedoya*², *M.C. Bortolini*^{3,4}, *A. Ruiz-Linares*^{2,4}. 1) Servicio de Hematologia, Facultad de Medicina, Universidad de Antioquia, Medellin, Colombia; 2) Laboratorio de Genetica Molecular, Facultad de Medicina, Universidad de Antioquia, Medellin, Colombia; 3) Departamento de Genetica, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil; 4) Department of Medical and Community Genetics, Imperial College School of Medicine, UK.

The sickle cell mutation was introduced in the American continent mainly by gene flow from Africa during the slave trade in the 15th-19th centuries. This mutation occurs in different haplotypic backgrounds and these have been used to trace the origin in Africa of the individuals brought to America. To examine the African ancestry of individuals introduced in Colombia we examined five RFLPs (HincII/e, XmnI/Gg, HindIII/Gg, HindIII/Ag, HincII/3'yb) in the b-globin gene cluster. Seventy eight unrelated sickle cell patients and an additional twenty heterozygote individuals (HbAS) were investigated, with a total of 176 HbS chromosomes available for study. The b-S haplotype frequencies observed were: Bantu (51.5%); Benin (42%); Senegal (3%); Cameroon (2%); other (1.5%). Previous studies have shown that the Benin haplotype is the most frequent (51-74%) in the US and in several Caribbean populations (Cuba, Jamaica, Guadeloupe, Venezuela). Despite being located on the Caribbean, our findings in Colombia differ from those results in showing the highest frequency for the Bantu haplotype and a relatively lower frequency for the Senegal haplotype. We are currently assessing the role of drift and population substructure in explaining the observed differences. As has been suggested for Brazil, b-S haplotype frequencies in Colombia could indicate that a relatively important fraction of African individuals introduced in this country during colonial times originated in Angola. For the US and in most Caribbean Countries an origin in West African seems predominant.

The association between low density lipoprotein receptor-related protein (LRP) and Alzheimer's disease is modified by apolipoprotein E and alpha-2 macroglobulin. *J.M. Kwon¹, S. Chakraverty², M. Martinez², J.C. Morris¹, A.M. Goate².* 1) Dept Neurology, Washington Univ, St Louis, MO; 2) Dept Psychiatry, Washington Univ, St Louis, MO.

Alzheimer's disease (AD) is etiologically complex, with genetic factors playing an important role. Mutations in three genes, amyloid precursor protein (APP), presenilin-1 gene, and presenilin-2 gene are known to cause early onset familial AD. These genes, taken together, probably directly cause 1% of AD cases overall. Apolipoprotein E remains an important susceptibility gene, accounting for nearly 40-50% of the genetic risk for developing AD. Recent linkage studies of late-onset AD suggest that another susceptibility locus lies on chromosome 12. Two candidate genes on chromosome 12, low density lipoprotein receptor-related protein (LRP) and alpha-2 macroglobulin (A2M) are biologically relevant to the development and pathogenesis of AD. While a number of association studies have been done to determine whether the polymorphic variants of these two genes are associated with AD, the results have been contradictory. No study, however, has looked at the joint effects of the two genes, though A2M is a ligand of LRP. We have looked at A2M and LRP variants in a large number of sporadic AD cases and age-matched controls and found that they are not associated with AD in unstratified analysis. However, the 5' tetranucleotide polymorphism of LRP and the exon 18 polymorphism of A2M are both associated with AD in individuals who are e4 carriers. In addition, the degree of LRP association with AD is modified by the A2M genotype. Our data suggest that it is the interaction between the genetic variation in LRP and A2M which confers risk for AD.

Linkage and Association Analyses of Sequence Variants of the GABRA6, GABRB2, and GABRG2 gene cluster and Alcohol Dependence in a Finnish Sample. *M. Radel¹, R.L. Vallejo¹, N. Iwata¹, R. Aragon¹, H. Naukkarinen², M. Virkkunen², J.C. Long¹, D. Goldman¹.* 1) Laboratory of Neurogenetics, DICBR/NIAAA/NIH, Rockville, MD 20892; 2) Department of Psychiatry, University of Helsinki, Finland.

In an autosomal genomic scan in Finnish pedigrees, evidence for linkage to alcohol dependence was observed at the long arm of chromosome 5 coinciding with the location of a cluster of GABAA receptor subunit genes, which include GABRA6, GABRB2, and GABRG2. In this study, the role of GABRA6, GABRB2, GABRG2 in alcoholism in a data set from Finland was evaluated by sib-pair linkage analysis. In addition, association between variant alleles and alcohol dependence was evaluated by comparing genotypic and allelic frequencies between unrelated cases and controls from the same population. The 473 psychiatrically interviewed Finns included 89 alcoholic offender probands, 277 relatives and 107 unrelated controls. There were 73 concordant sib pairs for alcoholism (DSMIII-R alcohol dependence), 67 discordant, and 98 unaffected. We genotyped six single nucleotide polymorphisms (SNPs) of the chromosome 5 long arm GABAA gene cluster (i.e., GABRG2 IVS8+99, GABRG2 IVS9+3415A>G, GABRA6 1031G>C, GABRA6 1236 C>T (Pro385Ser), GABRA6 1519T>C, and GABRB2 1412C>T) by PCR-RFLP analysis. Hardy-Weimberg equilibrium, as well as differences in allelic and genotypic frequencies were tested by Chi-square analysis and Fishers exact test (one and two tailed analyses). Sib-pair regression was performed with the Sibpal module included in S.A.G.E. (1996). Genotypic distributions were in Hardy-Weimberg equilibrium and did not differ significantly between cases and controls. Sib-pair linkage of GABRG2 to alcohol dependence was observed ($P=0.034$). There was no evidence of association between GABRG2 and alcohol dependence in our samples. Taken in conjunction with the evidence mentioned above, these results are consistent with genetic variation at or near the GABAA gene cluster on chromosome 5 playing a role in differential susceptibility to alcohol dependence.

Genomic control for association studies: A semiparametric test to detect excess haplotype-sharing. *K. Roeder¹, L. Wasserman¹, W. Byerley², B. Devlin³*. 1) Dept Statistics, Carnegie Mellon Univ, Pittsburgh, PA; 2) Dept. Psychiatry, University of California, Irvine; 3) Dept. Psychiatry, University of Pittsburgh, Pittsburgh, PA.

Individuals who share a disease mutation from a common ancestor often share alleles at genetic markers adjacent to the mutation even if the common ancestor is remote. Ideally, for a sample of affected individuals, this dependence -- measured as haplotype-sharing -- will be greater in the vicinity of disease genes than in other regions of the genome. We present a semiparametric test for haplotype-sharing. We begin by developing a model assuming that the ancestral haplotype is known and thus the extent of haplotype-sharing from a common ancestor can be determined unambiguously. The amount of overlap at markers far from the disease is treated as a random variable with an unknown distribution F , which we estimate nonparametrically. Overlap of markers surrounding disease genes are modeled as a mixture, $pF(x-f) + (1-p)F(x)$, in which p is the fraction of subjects with the disease mutation and f is the average excess overlap with the ancestral haplotype for that fraction of haplotypes that have the disease mutation. Testing for a disease gene then amounts to testing whether $p=0$. Next we drop the assumption that the ancestral haplotype is known. To detect excess clustering of haplotypes, we measure the pairwise overlap of a set of haplotypes. As in the simpler scenario, this distribution is modeled as a location-shift mixture. To test the hypothesis we construct a score test with a simple limiting distribution. These ideas are illustrated using a statistic designed for a less refined grid of markers. The data are a sample of schizophrenics and controls from the Remote Oceanic population of Palau.

Development of a DNA-diagnostic protocol for X-linked Mental Retardation (MRX). *H.G. Yntema, A.O. Oudakker, J. Theelen, E. Sistermans, E.C.M. Mariman, D.F.C.M. Smeets, H.G. Brunner, B.C.J. Hamel, H. van Bokhoven.* Dept Human Genetics 417, Univ Hosp Nijmegen, Nijmegen, Netherlands.

The high prevalence of mental retardation (MR) in the population poses an important medical and socio-economical problem to our society. In spite of extensive medical investigations, in most patients the etiology of the mental handicap remains unexplained. It has been estimated that up to 25%; of all MR in males is caused by X-chromosomal gene defects (MRX). Eight MRX genes have been identified in the last four years, but in each of these genes in only 1-2%; of the patients tested the underlying genetic defect has been found. Since more than 70 different linkage intervals on the X-chromosome have been identified, it can be estimated that 50 or more MRX genes exist. Together with research groups in Leuven, Paris, Tours and Berlin we participate in the European X-linked Mental Retardation Consortium. Due to this collaboration we all have access to more than 200 well-characterized families. The consortium participated actively in the identification of five of the MRX genes identified so far: *GDII*, *OPHN1*, *IL1RAPL*, *TM4SF2*, and *aPIX*. To identify new MRX genes, we are performing a high-throughput mutation analysis in X-chromosomal candidate genes by direct sequencing of PCR amplified exons in the cohort of 200 families. Newly identified genes and known MRX genes that are found to be most frequently mutated in the 200 MRX families, will subsequently be tested in a cohort of 500 males with unexplained MR. This work will allow us to determine exactly the contribution of each gene to the incidence of mental retardation in the west-European population. The only diagnostic test currently available is for Fragile X Syndrome, which explains approximately 5%; of all MR. By determining the contribution of other MRX genes, we intend to establish a cost-effective DNA-diagnostic protocol. Worldwide use of such a protocol should at least double the number of etiologic diagnoses in males with MR.

Genetic epidemiology of malaria infection in the Western Amazon region. *F.P. Alves¹, M.M. Moura^{2,3}, V. Engracia^{2,3}, M.F. Feitosa⁴, L.H. Silva³, E.P. Camargo^{1,3}, B. Beiguelman¹, H. Krieger^{1,3}.* 1) Instituto de Ciências Biomédicas, Univ. São Paulo, SP, Brazil; 2) Universidade Federal de Rondonia, RO, Brazil; 3) Centro de Pesquisas Medicina Tropical, RO, Brazil; 4) Washington University School Medicine, MO, USA.

Malaria, the world's most important cause of infectious morbidity and mortality has a widespread geographical distribution while its endemicity is very heterogeneous. The Amazon region, due mainly to its relatively low demographic index, is characterized as an oligo-endemic region. A population of 182 individuals, living in Portuchuelo, State of Rondonia, Brazil, right bank of the Madeira river (8°37' S, 63°49'W) was surveyed, in order to ascertain data on the epidemiological aspects of malaria and some other infectious diseases. Two main phenotypes involved with *Plasmodium* infection were studied: a) the presence of asymptomatic infection *i.e.*, presence of *Plasmodium*, diagnosed by traditional thick smear and/or by PCA amplification of *Plasmodium* ribosomal DNA in blood samples; b) the reported number of previous malaria episodes. Segregation analysis using the unified model of Lalouel et al. (1983) was applied to both phenotypes. There were no evidences of any type of familial mechanisms acting on the distribution of the asymptomatic phenotype in this population, since there was no significant sign of familial aggregation for this trait. As for the number of malarial episodes, this phenotype showed clear signs of Mendelian inheritance. The most parsimonious model includes a co-dominant major gene with frequency of $q = 0.15$ and a small multifactorial component ($h = 0.08$). This finding seemed to be independent of the Hb, Fy or G-6-Pd polymorphisms. (FAPESP, CNPq, CAPES).

Heritability of age at death in the Old Order Amish. *B.D. Mitchell¹, W.-C. Hsueh¹, T.M. King^{2,3}, T.I. Pollin⁴, J. Sorkin⁴, A.R. Shuldiner⁴.* 1) Southwest Foundation for Biomedical Research, San Antonio, TX; 2) UT MD Anderson Cancer Center, Houston, TX; 3) Genometrix, Inc., The Woodlands, TX; 4) University of Maryland School of Medicine.

There are approximately 30,000 Old Order Amish (OOA) currently living in Lancaster County, PA, virtually all of whom can trace their ancestries back to a small number of descendants who settled in the area between 1727 and 1780. Extensive genealogical information, including birth and death dates, has been maintained throughout this time, thus providing the opportunity to evaluate the familial components of age at death in this unique population. Our analyses were based on 1,655 individuals, representing all those born prior to 1890, surviving until at least age 30, and with known date of death.

Mean age at death (\pm s.d.) in this population was 70.7 ± 15.6 yrs, and this did not change appreciably over time. Mortality rates were similar between men and women, except at ages 30-50, during which time mortality rates were slightly higher in women than in men. There was a strong association between parental and offspring age at death. Mean age at death was 69.4 ± 15.3 yr. in individuals for whom both parents died before the age of 75 ($n = 280$), 71.5 ± 15.1 yr. in individuals for whom one parent died before the age of 75 ($n = 567$), and 73.5 ± 16.0 yr. in individuals for whom neither parent died before the age of 75 ($n = 311$). These differences were highly significant ($p = 0.006$). Using maximum likelihood methods, we estimated heritability of age at death by partitioning the phenotypic variance into genetic and non-genetic sources. From these analyses we estimated that additive genetic factors accounted for 25% ($\pm 5\%$) of the total variance in age at death. We conclude that longevity is moderately heritable in the OOA.

Pseudodominant transmission of hereditary hemochromatosis in a family from a small geographic area: genealogical and genetic analysis. *L. Mota-Vieira¹, P. Macedo¹, C. Paiva¹, L. Serpa¹, H. Brandao¹, J. Almeida-Mello², P. Rodrigues³, G. Porto³.* 1) Hospital Divino Espirito Santo, Ponta Delgada, Azores; 2) Azores Genealogical Club; 3) Molecular Immunology Unity - IBMC, Porto, Portugal.

In a small village of the Azores (Portugal) we identified one family with vertical inheritance pattern of hereditary hemochromatosis (HH), an inborn error of iron metabolism that leads to progressive iron overload. The HH is an autosomal recessive disorder frequent among Caucasians but rare in the Azorean population. Homozygosity for a C282Y mutation in the hemochromatosis (*HFE*) gene is the underlying defect in 80% of HH patients. Based on clinical study and family history we identified 6 affected individuals in 3 consecutive generations: the paternal grandfather in the 1st generation, the father and a paternal uncle and aunt in the 2nd generation, and the proband and his brother in the 3rd generation. These affected siblings have the HH classical profile, including all biochemical variables related to iron metabolism. However, their uncle has a minor form of HH with only increased serum ferritin and transferrin saturation. In order to understand the HH pseudodominant transmission in this family, we performed a genealogical study and an *HFE* mutation screening. Based on church registers, civic records and pedigree analysis we identified 4 consanguineous marriages and 2 distinct surnames that can be related to the disease. The molecular analysis revealed homozygosity for the C282Y (+/+) mutation in 3 HH patients (proband, his brother and his uncle) and in 1 asymptomatic proband sister, and detected 6 carriers for the same mutation (+/-). Due to geographic isolation and small size of the village (560 inhabitants), we performed genotyping of the spouses, homozygous and carriers, in order to be more selective in the investigations of their children.

This study suggests that in this family the HH transmission is due to the segregation of three C282Y alleles, most probably having the same chromosomal background. To test this hypothesis, the haplotype analysis is being performed, in association with the search for a unique founder couple or for common links between surnames.

Recurrence risks of stuttering in family relatives and their genetic implications. *N.S. Viswanath¹, H. Lee², J.P. Alexander¹, D.B. Rosenfield¹, R. Chakraborty².* 1) Stuttering Center Speech Motor Control Lab., Dept. Neurology, Baylor College of Medicine, Houston, TX; 2) Human Genetics Center, Univ. Texas School of Public Health, Houston, TX.

Stuttering is an early childhood speech motor disorder characterized by repetition and prolongation of sounds and syllables and blockage of flow of speech. It affects all aspects of individual's life, as speech is the primary mode of communication in the human society. For this reason, multiple epidemiological as well as genetic studies have been undertaken to characterize the etiology of stuttering. General prevalence of stuttering is about 1% or less in most Western populations, with a conspicuous sex dimorphism (3:1 male-female ratio). Concordance rate in monozygotic twins is significantly higher than that of dizygotic twins, and family relatives of stutterers have a significantly higher recurrence risk than that in the population. In spite of these results, the mode of inheritance of stuttering still remains controversial. We collected 56 multigenerational families, each ascertained through a single proband (38 male and 18 female), from which information about stuttering were collected on 1235 blood relatives, and 326 other family members married into the families. These data show that the biological relatives of stutterers have a nearly 10-fold higher risk than that in the population and recurrence risks in relatives decrease with the degree of kinship. Recurrence risks for male relatives are greater than for female relatives in each kinship category. However, recurrence and relative risk rates are not affected by the proband's sex. This last observation is incompatible with a polygenic threshold model of inheritance. Decay of recurrence risks with the kinship coefficients of relatives is not consistent with a simple monogenic mode of inheritance either. Complex segregation analysis is underway to better elucidate the mode of transmission and to detect pedigrees for subsequent linkage analysis to locate gene(s) for susceptibility of stuttering. (Research supported by M. R. Bauer Foundation and by the US Public Health Service Research grant GM 41399 from the National Institutes of Health).

Background linkage disequilibrium in 18q21-q22 in the Finnish and Costa Rican populations. *L.N. Bull¹, S.K. Service², A.-E. Lehesjoki³, A. de la Chapelle⁴, M. Slatkin⁵, N.B. Freimer²*. 1) Liver Center Laboratory, Dept. of Medicine, SFGH, UCSF, SF, CA; 2) Neurogenetics Laboratory, Dept. of Psychiatry, UCSF, SF, CA; 3) Folkhälsan Institute of Genetics, and Dept. of Medical Genetics, Haartman Institute, Helsinki, Finland; 4) Comprehensive Cancer Center, Ohio State U., Columbus, OH; 5) Dept. of Integrative Biology, UC Berkeley, Berkeley, CA.

We investigated the distribution of background linkage disequilibrium in multiple populations, using 26 polymorphic microsatellite markers in a well-mapped 22 cM region on chromosome 18q21-q22. We typed these markers in a Finnish population sample containing 96 chromosomes, and in a population sample from the Central Valley of Costa Rica containing 54 chromosomes. Half the individuals in the Finnish sample are from a population founded ~2000-2500 years ago, and the other half, from a population founded ~400 years ago. The current population of the Central Valley of Costa Rica was founded 300-400 years ago; little subsequent immigration occurred until quite recently. LD was assessed using the Fisher's exact test, applied to the data from the two Finnish samples separately and together, and also to the Costa Rican sample. In the younger Finnish sample, in each of the 5 marker pairs with LD $p < 0.01$, the markers are within 2 cM of each other. In the older Finnish and Costa Rican samples respectively, markers in 4 of the 12 pairs and 4 of the 10 pairs with LD $p < 0.01$, are > 2 cM apart. The Mantel test was applied to data for all marker pairs to test for evidence of a relationship between LD and genetic distance, and evidence was found in all four analyses (p-values ranged from < 0.00001 to 0.015). The LD p-value between markers increased between 0.0066 and 0.013 for every increase of 1 cM in genetic distance, depending on the sample. The rate of decay of LD with increasing genetic distance appears faster in the younger, than in the older, Finnish sample, and slowest in the Costa Rican sample.

Creating an LD map of a long genomic region. *M.J. Daly, J.D. Rioux, E.S. Lander.* Whitehead Institute/MIT Center for Genome Research, Cambridge, MA.

We have developed a conceptual framework for elucidating and visualizing ancestral haplotypic diversity in densely SNP genotyped genomic regions. The basic concept is the identification of short blocks of DNA in which historical recombination has played little or no role creating diversity. These blocks can, for all practical purposes, be considered single loci and the linear connection of these blocks (across intervals in which recombination has played an assortative role) over long genomic regions provides an "LD map" of the entire genomic region. This map allows one to specify a greatly reduced subset of SNPs from a dense genomic region that together fully explain the ancestral haplotype diversity in the region for genotyping in subsequent samples. Having created such a representation of several regions, it will further allow us to estimate a general density of random SNPs that would be needed in unexplored genomic regions to achieve the same information. Additionally, once identified, the blocks themselves become extremely powerful tools. Treating them as single multi-allelic loci enables more powerful and accurate tests of linkage disequilibrium than may be offered by any of the component SNPs individually. Missing and ambiguous genotypes within a block may be filled in with very high accuracy since the diversity within any block is fully characterized. The application of the method is presented using data from several high density SNP genotyping projects ongoing at the MIT Genome Center.

Low linkage disequilibrium at the dopamine D4 receptor (DRD4) gene in the telomeric region of chromosome 11.

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The DRD4 gene is near the telomere in 11p15.5. The protein product has a high affinity for the neuroleptic Clozapine and localizes in GABAergic neurons. There is debate about whether polymorphisms in this gene are implicated in personality traits and psychiatric disorders. We typed samples from 32 globally distributed populations for two SNP polymorphisms in the 5' UTR of DRD4. One consists of 2 adjacent bases (-616,-615) with 3 haplotypes (CA,GA,GG) and is 100bp from the other site (-514). Both were polymorphic in all populations (estimated heterozygosity, .27-.66 and .27-.50, respectively). One of these polymorphisms (-615A/G) has not previously been reported. We also incorporated the data for 2 polymorphisms we previously reported--a 120bp tandem duplication in 5'UTR about 1 kb upstream, and the 12bp duplication in exon 1 about 1kb downstream (Am J Med Genet, 1999, 88:705-9). Haplotype frequencies and linkage disequilibrium (LD) were analyzed for these four sites spanning a 2 kb region. African populations have more haplotypes and higher heterozygosities, ranging up to .94, than most other populations, but heterozygosity is greater than .65 even in the populations with the fewest haplotypes. By a permutation test, overall LD is nominally significant only in some East Asian populations, in the one Melanesian sample, and in most Native American groups, but in the context of multiple tests only the LD in the Melanesian sample remains significant. In finding that LD per kb at this telomeric locus is greatly reduced compared to that in non-telomeric loci (such as DM, DRD2, PAH, CD4), we show that the value of association studies based on one marker at DRD4 is severely limited. We gratefully acknowledge the help of A.T. Quinones, J.R. Kidd, A.J. Pakstis, and J. Fisher in collection and analysis of these data. This work was supported in part by MH30929, GM57672, and a NARSAD grant.

Linkage disequilibrium in the ADH gene cluster. *M.V. Osier, A.J. Pakstis, J.R. Kidd, K.K. Kidd.* Genetics, Yale Univ, New Haven, CT.

The ADH gene family consists of seven known genes in a "cluster" of undetermined molecular extent on 4q21-24; the three class I genes are evenly spaced in 80 kb of that cluster, and are the best studied because two of them, ADH3 and ADH2, have SNPs resulting in amino acid substitutions yielding enzymes with different efficiencies metabolizing ethanol. We have previously demonstrated that the functional variation at ADH2 (exon 3) contributes to substantially different levels of protection against alcoholism in a case-control study of Chinese Han samples but that the functional variation at ADH3 (exon 8) does not have a detectable effect (Osier et al, 1999, AJHG,63:1147-1157). Understanding linkage disequilibrium (LD) between the variants at the two loci was crucial to that analysis. Because other functionally relevant variation may exist at these and/or other loci in the ADH family, we are extending LD studies to more sites and additional populations. To date we have identified three new SNPs (ADH3 intron 2 and exon 5, ADH6 intron 2), converted one RFLP to PCR (ADH2 intron 3), and typed these and the two functional SNPs (ADH3 exon 8, ADH2 exon 3) in samples from 31 populations (mean 2N=101.0). Allele frequencies of the ADH3 and ADH2 sites are strikingly different in the East Asian samples from those in samples from all other parts of the world: F_{st} values for the three non-functional SNPs in the Class I genes decrease from 0.10,0.20,0.79 (across all 31 populations) to 0.07,0.16,0.14 (across 24 populations) when the East Asian samples are removed. There is strong evidence of LD across these five sites ($0.58 < x < 4.08, p < 0.01$). The SNP at ADH6 is an unknown distance away and does not show a clear pattern of LD with the five Class I SNPs. Using a segment version of a permutation test comparing the ADH6 SNP to the haplotypes of the Class I SNPs gives nominally significant evidence of LD in 6 population samples (1 African, $p < .05$; 1 European, $p = .004$; 1 East Asian, $p < .05$; 2 North American, $p < .05$; and 1 South American, $p < .001$), while one would expect at most two by chance. Recent, population-specific factors, such as random drift or selection, would seem the most likely explanation since no regional or global pattern is evident. Supported by AA09379.

The extent of linkage disequilibrium in European Americans. *F.J. McMahon¹, Y.-S. Chen¹, M.G. McInnis², C. Yarnes¹.* 1) University of Chicago, Chicago, IL; 2) Johns Hopkins University, Baltimore, MD.

The success of SNP-based linkage disequilibrium (LD) mapping may depend on the extent of LD between adjacent markers, but little is known about the extent of inter-marker LD in non-isolated human populations. Preliminary to SNP-based studies, we have mapped 11 chromosome 18q microsatellite markers on the Stanford TNG radiation hybrid panel at estimated mean inter-marker distances of 237 kb. These markers were genotyped in a set of 58 European-American pedigrees collected for a linkage study of bipolar disorder. Genotypes were generated using semi-automated fluorescent methods and assembled into haplotypes. Haplotype data was analyzed for inter-marker LD in the founders by use of a Monte Carlo permutation of the Fisher Exact Test. Significant ($p < 0.001$) LD was observed between 6 of 10 adjacent marker pairs and 11 of all 55 possible marker pairs. Eight markers showed significant LD with at least one other marker. The distances between markers in LD were significantly smaller than the distances between markers showing no detectable LD. Significant LD was detected between 3 different marker pairs with an inter-marker distance of >450 kb. These results demonstrate that linkage disequilibrium can be detected over relatively large physical distances on chromosome 18q in European Americans who share a common complex genetic disease.

Generation of linkage disequilibrium in an African American population by recent gene flow from diverse populations. *R.A. Kittles, C. Rotimi, G. Bonney, G. Dunston.* National Human Genome Center at Howard University, Washington, DC.

The African American population is a heterogeneous macro-ethnic group due to its unique history. Because of this history, increased attention has focused on the African American population for disease gene mapping studies that take advantage of admixture linkage disequilibrium (LD). While most studies have focused on admixture with European Americans, sparse data exist about the contribution other populations, including Africans, have had on generating LD in the population. Here we evaluated genetic contributions from different populations by examining haplotypic variation for two genes. African American (n=520) samples were from Columbia, SC. West African samples were from Ghana (n=95), Liberia (n=75), Nigeria (n=83), Senegal (n=85), Sierra Leone (n=180) and the Cameroon (n=70). For non-African comparisons, European Americans (n=87), Asians (n=60), and Amerindians (n=80) were included. Haplotypes were constructed using three polymorphic markers (1 SNP and 2 STRs) separated by 1 kb within exon 1 of the androgen receptor gene (AR), and three markers (2 SNPs and an STR) separated by 40 kb within the 5-alpha reductase gene (SRD5A2). While the high mutation rate may have randomized alleles at the STR loci, significant disequilibrium was observed between markers for both genes in African Americans and Amerindians. A common AR haplotype was shared among all non-African populations however, for 4 of the 6 African populations the most common haplotype was different. Of the significant allelic associations between AR markers in the South Carolina population, 26% originated from European Americans, 13% from Ghanaians, 26% from Sierra Leone, and 26% were shared among west African populations. Although a similar pattern of haplotypic variation was observed for the SRD5AR locus, significant differences in levels of LD was observed for several of the non-African populations. These results reveal that the LD generated in African American's from Columbia, SC may be due to recent migration of African Americans from diverse rural communities following urbanization, recurrent gene flow from distinct West African populations, and admixture with European Americans.

Conflicting estimates of the population recombination rate. *M.F. Przeworski*¹, *J.D. Wall*². 1) Statistics, Oxford University, Oxford, United Kingdom; 2) Dept. of Org. Evol. Biol., Harvard University, Cambridge, USA.

The efficient design of association mapping studies relies on a knowledge of the rate of decay of linkage disequilibrium with distance. This rate depends on the population recombination rate, C . An estimate of C is usually obtained from a comparison of physical and genetic maps, assuming an effective population size of approximately 10,000. We demonstrate that, under both a constant population size model and a model of long-term exponential growth, there is evidence for more recombination in polymorphism data than is expected from this estimate. An intriguing possibility is that the majority of meiotic recombination events are gene conversion without exchange of flanking markers.

Linkage Disequilibrium in Isolated and Outbred Chinese Populations. *J. Xiao^{1,2}, J.M. Akey², M.M. Xiong², L. Jin^{1,2}*. 1) Inst Genetics, Fudan Univ, Shanghai, China; 2) Human Genetics Center, University of Texas, Houston, TX.

The proposition of genome wide linkage disequilibrium (LD) screens has stimulated considerable optimism in identifying genes underlying complex diseases. Moreover, many investigators have advocated the use of isolated populations because LD is predicted to extend over larger chromosomal distances. However, LD is a complex population genetic phenomenon, which is affected by many parameters including demographic history, selection, mutation, genetic drift, and recombination rates. Thus, the genomic distribution of LD and its inter-population characteristics are largely unknown. If LD screens are to become a practical and useful tool then theoretical predictions based on simple population genetic models need to be empirically verified. To this end, we have genotyped male individuals in five isolated (average $n=96$) and one outbred Chinese population ($n=110$) for both 11 microsatellite and 13 SNP markers spanning Xq22-q25. Using a variety of LD measures and statistical tests such as D , D' , r^2 , and Fishers Exact Tests, we show that 3 out of the 5 isolated populations have quantitatively more LD than the outbred population. Hence, it is unwise to assume all isolated populations offer advantages in LD mapping, and underscores the benefit of preceding a gene mapping experiment by a population genetic study. Finally, our data allows us to address several other interesting topics that will be discussed such as: 1) the advantages of multilocus haplotypes in LD analyses, 2) the difference in "LD maps" depending on the type of marker used to create them, and 3) the utility of haplotypes comprised of a mixture of SNPs and microsatellites markers.

Linkage Disequilibrium Mapping Using Genotype Data. *S. Zhang, H. Zhao.* Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT.

Linkage disequilibrium mapping (LDM) has proven a powerful tool for fine-mapping disease genes. Statistical methods that use multiple markers simultaneously are more powerful than those using markers separately. However, existing multilocus methods all assume that haplotype information is available for statistical analysis. But haplotypes are often difficult to obtain, especially for studies involving many genetic markers and relatively few individuals from each pedigree. To overcome this apparent discrepancy between statistical methods and genetic data that are available to geneticists, we have developed a multilocus approach for LDM using genotype data. Because disease models are usually unknown for complex traits of interest, our model introduces a heterogeneity parameter to capture differences among different disease models. Because this heterogeneity parameter is estimated together with other parameters (e.g. disease gene location and age of mutation) in our analysis, our approach does not depend on any prior knowledge of the disease model. In our maximum likelihood estimates of the model parameters, we treat the unobservable haplotypes as complete data, and the observable genotypes as incomplete data, and use the expectation-maximization algorithm to estimate the model parameters. For haplotype analysis, we employ the Decay of Haplotype Sharing (DHS) method proposed by McPeck and Strahs [1999] and extended by us (Zhang and Zhao 2000). We assess the performance of our methods through extensive simulations under a variety of disease models, population history, sample sizes, and genetic marker spacing. Our simulation results suggest that the methods are very robust for locating disease genes, even for small sample sizes.

The genome-wide distribution and extent of background linkage disequilibrium in a population isolate. *S.K. Service, R. Ophoff, N.B. Freimer.* Neurogenetics Lab, Univ California, San Francisco, CA.

Recent interest in using association studies to elucidate the genetic basis of complex traits has focused much attention on determining the extent of linkage disequilibrium (LD) in anonymous regions of the human genome. We examined the genome-wide distribution and magnitude of LD using a set of densely and evenly spaced microsatellites (n=1036), in a sample from a genetically homogeneous and demographically well-characterized population, that of the Central Valley of Costa Rica. As we were interested in background LD (BLD) rather than disease-associated LD, we analyzed 157 control chromosomes from a disease-mapping study. High levels of BLD were found between linked markers in the sample, with 61% of markers within one cM of each other in significant BLD ($p < 0.05$). Although BLD was significantly related to genetic distance between markers (with marker pairs four cM apart having twice the odds of being in BLD than markers more than four cM apart), it was not spread uniformly throughout the genome and was observed for numerous marker-pairs separated by distances of up to about seven cM from each other. We discuss the implications of these findings for LD mapping, particularly in population isolates.

Distribution of Bardet-Biedl syndrome loci among 51 North American/European, Kurdish and Pakistani

pedigrees. *P.L. Beales¹, E.N. Katsanis¹, R.A.L. Lewis^{1,2}, J.R. Lupski¹*. 1) Dept Molecular & Human Genetics, Baylor College Medicine, Houston, TX; 2) Cullen Eye Institute, Baylor College Medicine, Houston, TX.

Bardet-Biedl syndrome (BBS), an autosomal recessive disorder, is characterized by RP, polydactyly, obesity, mental retardation, hypogonadism, and renal dysplasia. Neither the underlying genetic nor the pathophysiological defect is known but five BBS loci have been mapped: BBS1-5 (11q13, 16q21, 3p12-13, 15q23, 2q31). Positional cloning efforts have thus far proven ineffective. Previously, we reported refinement of the critical interval for BBS1, accounting for 40% and report here the results of assignment by haplotype analysis to the remaining 4 loci. Of 51 pedigrees excluded from 11q13, 30 were North American/ European Caucasian, 21 were Kurdish/Pakistani. All pedigree members were genotyped with fluorescent microsatellites from the 4 loci. Analysis of the outbred Caucasian families determined that 43% (17% were unassignable) were linked to at least one locus with the following distribution: 7 pedigrees (23%) to 16q21; 3 pedigrees (10%) to 2p31; 2 pedigrees (7%) to 3p13; and 1 pedigree (3%) maps to 15q23. 40% were unlinked. When 11q13 is included, the overall relative distribution for 5 loci is: BBS1 39%, BBS2 15%, BBS3 4%, BBS4 2%, BBS5 6%, Unlinked 23%; (11% unassignable). 2 BBS2 pedigrees had recombinants, which reduced the published critical interval from 18 cM to 2.5 cM. A BBS3 pedigree reduced the interval from 6 cM to 4 cM. The second study comprised 13 Kurdish, 7 Pakistani and 1 Indian families. We assumed that given the consanguinity of each family, we need only look for identity-by-descent (IBD) to determine potential linkage. 5 families (24%) were IBD for all markers from 15q23 (BBS4); 3 were of Kurdish origin and 2 Pakistani. One Pakistani family was IBD at 16q21 (5%); a further pedigree was IBD for both BBS2 & 4 regions. Given that no other pedigree showed dual IBD, this may represent digenic inheritance, a hypothesis that may eventually prove to be common for BBS. Overall, 7% (16) of non-Caucasian pedigrees were non-IBD for all loci, suggesting the presence of a further BBS locus (or loci). This study has enabled us to move closer to positional cloning of BBS.

Accurate estimation of the recombination fraction for complex disorders using lod scores. *M.W. Logue, V.J. Vieland.* University of Iowa, Iowa City, IA.

It has long been known that lod scores (LODs) based on misspecified models can result in biased estimates of the recombination fraction. Yet accurate estimation of q is critical to fine mapping and comparison of results across multiple studies. Here we begin to test the hypothesis that the bias observed in q in some studies has resulted from the practice of fixing the parameters of the trait model when maximizing the LOD, rather than estimating them. We illustrate this with calculation of the asymptotic m.l.e. of q based on the heterogeneity lod (HLOD) for sibships of size 2. We model two recessive disease loci, only one of which is linked to the marker, with penetrances f_1 and f_2 for the linked and unlinked families respectively. For this model, the HLOD is misspecified and subject to ascertainment bias. The Table below shows typical results for one model, with true $q=0.05$, $f_1=0.7$, $a=.6$, and truncate ascertainment. For different values of f_2 , ELODS are maximized over a and q , with either f_1 fixed at its true value or with f_1 estimated as well.

Estimate of q for a given F_2

$f_2=0.2$ $f_2=0.4$ $f_2=0.6$ $f_2=0.8$ $f_2=1$

f_1 Fixed 0.057 0.057 0.052 0.048 0.046

f_1 Est. 0.050 0.050 0.050 0.050 0.050

When f_1 is fixed, even at its correct value, the estimate of q is biased as a function of the unknown f_2 , except in the case $f_1=f_2$ for which the HLOD is correctly specified, But estimation of f_1 allows asymptotically unbiased estimation of the genetic distance. Simulations are needed to extend this result. We conclude, however, that by estimating parameters of the genetic model q can probably be accurately measured even for complex disorders.

Maximum likelihood based linkage disequilibrium mapping for two-marker haplotype data with comparison to a composite likelihood approach. *C. Garner, M. Slatkin.* Department of Integrative Biology, University of California, Berkeley, CA.

We report a theory that gives the sampling distribution of two-marker haplotypes that are linked to a rare disease mutation. The method uses a Monte Carlo approximation in order to estimate the location of the disease locus. We present a comparison of the performance of this method to a composite likelihood of single loci statistics. Through simulation, we show that the composite likelihood provides a good approximation to the maximum likelihood, in general. Haplotype configurations exist for which the composite likelihood will fail to place the disease locus in the correct marker interval. For example, low levels of linkage disequilibrium between the markers and disease locus can lead to the disease locus being falsely localized to outside of the markers when the true location lies between the markers. An assumption of composite likelihood is that the individual likelihoods are independent, this is not the case when the markers are in linkage disequilibrium and the confidence intervals can be different from their true values. A bias in the composite likelihood increases as the linkage disequilibrium between the markers increases; however, this bias is small for diallelic markers having alleles with low frequency. The new method is used to estimate the location of the gene for diastrophic dysplasia in the Finnish population.

Markov chain Monte Carlo oligogenic segregation analysis of familial prostate cancer pedigrees. *E.M. Conlon¹, E.L. Goode¹, M. Gibbs², J.L. Stanford², M. Badzioch¹, M. Janer³, S. Kolb², L. Hood³, E.A. Ostrander², G.P. Jarvik¹, E.M. Wijsman¹.* 1) Univ Washington; 2) Fred Hutchinson Cancer Res Center; 3) Institute for Systems Biology, Seattle, WA.

Previous studies have suggested strong evidence for a familial component to prostate cancer (PC) susceptibility. We analyze 3,796 individuals in 263 prostate cancer families recruited as part of the ongoing Prostate Cancer Genetic Research Study (PROGRESS). We use Markov chain Monte Carlo methods with simulations of size 500,000 for oligogenic segregation analysis to estimate the number of quantitative trait loci (QTLs) and their contribution to the variance in age at onset of PC. Run time is ~5.25 hrs using a DEC Alpha XP1000 workstation. We estimate that a mean of 4.81 QTLs make contributions to the variance in age at onset of PC, with an estimated 84.1% posterior probability of at least 4 QTLs contributing to the variance. Environmental effects contribute ~18% to the variance in age at onset, with genetic effects contributing the remaining ~82%. The largest QTL contributes ~61% to the genetic variance, the second largest contributes ~20%, the third largest contributes ~12%, and the remaining QTLs contribute in total less than 7% to the genetic variance. The largest QTL is estimated to be a dominant locus for the more common allele, with frequency of the more common allele in these families estimated to be 0.580 (0.17 SD). With the common homozygote fixed as reference, the rarer homozygote had an estimated effect of +15.3 yrs (19.3 SD). The second largest QTL is estimated to be overdominant, with the heterozygote genotype estimated to confer highest risk, although this could be an artifact of the ascertainment and/or analysis approach. The frequency of the more common allele is estimated to be 0.583 (0.18 SD). With the common homozygote fixed as reference, the heterozygote had an estimated effect of -5.1 yrs (7.6 SD), and the rarer homozygote had an estimated effect of +5.9 yrs (12.0 SD). These results suggest the need to consider a relatively high disease allele frequency, although allele frequency estimates may be biased upward due to ascertainment of these families for linkage analysis.

Adjusting for the non-independence of pairs in the conditional logistic model for affected-relative-pair linkage. *K.B Jacobs*. Epidemiology and Biostatistics, Rammelkamp Center for Education and Research, MetroHealth Campus, Case Western Reserve, Cleveland, OH.

The Olson (*Am. J. Hum. Genet.* 65:1760-1769, 1999) conditional logistic model for affected-relative-pair (ARP) linkage analysis provides a general and flexible method to test for genetic linkage under a variety of multi-locus models using information from all affected relative pairs. However, the model as proposed assumes independence of all relative pairs, which will not be the case for data from extended pedigrees, or, indeed, from sibships of size greater than two. Cordell et al. (*Am. J. Hum. Genet.* 66:1273-1286, 2000) note this will inflate type 1 error and recommend that significance levels be calculated by use of simulation. We confirm this by large-scale simulation over various pedigree structures and compute the null distributions. We then discuss several possible simulation methods and their computational and statistical effectiveness to compute empirical estimates of significance levels. While effective, simulation is computationally expensive and can be infeasible in some situations. Therefore, a weighting scheme is developed to approximately correct for the non-independence of pairs due to allele sharing at a single locus so that simulation is unnecessary.

Linkage analysis of multivariate dichotomous response with latent class models. A.A. Todorov, E. Rassmussen, J.K. Seth, P.A.F. Madden, A.C. Heath. Dept Psychiatry, Washington Univ Sch Medicine, St Louis, MO.

Many complex traits are assessed using multiple items/symptoms. One possible analytical strategy is to summarize the items into a single measure (e.g., taking total symptom count). An alternative is to perform a potentially more informative multivariate analysis, at the cost of increased analytical complexity. In the present, we compare the power of latent trait (LT) and latent class, (LC) models in detecting linkage to a set of symptoms using combined samples of sibpair and twin data. The LT model (the probit model being a special case) surmises an underlying continuum of disease severity. The LC model, on the other hand, assumes that individuals will fall into one of several classes that may or may not be ordered in terms of severity (e.g., *normal*, *type A*, *type B*, *Combined*). Classes distinguish one another by different patterns of symptoms. Both types of models, subject to identification constraints, allow the incorporation of covariates.

Our implementation of the LT model does not require that the liabilities of two sibs/twins be distributed bivariate normal. Rather, it provides a choice of marginal distributions and copulas with one parameter that indexes the strength of the association between sibs/twins liabilities. Let d_r be this parameter for pairs of type r ($=$ MZ, DZ, ibd 2, 1 or 0). We test for linkage by comparing the log-likelihoods of two model with respective constraints (i) $d_0 = d_1 = d_2 = d_{DZ} \leq d_{MZ}$; and (ii) $d_0 \leq d_1, d_{DZ} \leq d_2 \leq d_{MZ}$.

The parameters of the LC model are (i) the probabilities that two sibs with genetic relationship r will fall into classes a and b , respectively (P_{rab}) and (ii) that an individual in a given class will express a given symptom. We estimate all parameters using the EM algorithm, imposing symmetry and marginal homogeneity constraints as needed (e.g., $P_{rab} = P_{rba}$, in the absence of covariates that distinguish between sibs/twins). We test for linkage using linear constraints of the diagonal elements of the joint class distribution. P-values are determined using randomization tests.

Linkage-Disequilibrium Mapping of Common Disease Loci. *G. Gong, R.R. Recker.* Osteoporosis Research Ctr, Creighton Univ, Omaha, NE.

We developed a mathematical model to estimate q , the recombination fraction between marker and complex-trait loci. A disease-predisposing mutation is assumed to occur initially on the haplotype with allele A_1 of a diallelic marker. Assume the mutation causes the disease only in the presence of certain environmental factor(s) and/or allele(s) not linked to the marker locus. Let the probability of simultaneous presence of *such* environmental factors and alleles be I , the penetrance of the disease be c , the frequencies of A_1 and A_2 from a sample randomly selected from the general population be p_1 and p_2 , respectively, the disease prevalence rate be p_d , the frequency of the disease linked to A_1 be p_{1d} , and the age of the mutation be t generations. Then, $(1 - q)^t = (p_{1d} - p_1 p_d) I c / (p_2 p_d I c) = (p_{1d} - p_1 p_d) / (p_2 p_d)$. p_d and p_{1d} also represent the prevalence rates when the disease locus has recurrent mutation with a mutation rate m per generation (but the above equation is no longer applicable). If other environmental factors and/or alleles not linked to the marker locus can cause the same disease independently (with a *theoretical* prevalence rate x in the *absence* of the disease allele linked to the marker locus), then the overall disease frequency p_d' is $p_d + x - x p_d$, while p_{1d}' (the frequency of the disease associated with, and linked to, A_1) is $p_{1d} + x p_1 - x p_{1d}$. Let k be the correction factor for incomplete initial disequilibrium. Thus, $[1 - (m + q)]^t = k(p_{1d}' - p_1 p_d')(1 - m)^t / \{p_2 [p_d' + (1 - m)^t(1 - x) - 1]\}$. This equation is applicable to diseases with dominant, codominant, or overdominant inheritance. Knowledge of I , c , k , m and x in particular from other types of genetic studies may lead to accurate estimation of q . If they are ignored partially or totally, q will only be overestimated, and an upper limit of q can be obtained. Using this model and published data, we estimated the distance between an Alzheimer's disease (AD) locus and a marker locus at the apolipoprotein E gene. The distance is at most 35 kb assuming $t = 5000$ (the AD-apoE association is found in the three major races, suggesting that the disequilibrium predates the divergence of races 100,000 years ago). We thus established a simple model to map complex trait loci.

Mutations in Klotho are associated with survival in diverse human populations. D.E. Arking¹, A. Krebsova², M. Macek Jr.², M. Macek², A. Arking³, I.S. Mian⁴, L.P. Fried⁵, A. Hamosh¹, S. Dey¹, I. McIntosh¹, H.C. Dietz¹. 1) HHMI and Institute of Medical Genetics, John Hopkins SOM, Baltimore, MD; 2) Institute of Biology & Medical Genetics, Univ. Hospital Motol and 2nd SOM Charles Univ., Prague, Czech Republic; 3) Johns Hopkins University, Baltimore, MD; 4) Department of Cell & Molecular Biology, Lawrence Berkeley National Laboratory, Berkeley, CA; 5) Center on Aging & Health, JHMI, Baltimore, MD.

Mice homozygous for null alleles of the *klotho* gene exhibit a syndrome resembling human aging, including atherosclerosis, osteoporosis, emphysema, and short life span. Using marker allele data from a Bohemian Czech population, we have previously demonstrated that the KLOTHO locus is associated with aging. We have now characterized an allele defined by the presence of six mutations that occur within an 800 bp region spanning exon2 and flanking sequence that, in homozygosity, is associated with decreased survival. This observation has been confirmed in three independent populations: Bohemian Czech, Baltimore Caucasian, and Baltimore African American (combined OR = 2.59, $p < .003$). In the Bohemian Czech population, heterozygosity for this allele is associated with increased survival ($p < .04$). Of the three mutations in exon 2, two code for amino acid changes, F354V and C372S, and one is silent. To assess the potential functional significance of these amino acid variations we created a Hidden Markov Model (HMM) for the two glycosidase domains in Klotho (Klotho1 and Klotho2) and the glycosidase of known structure which is most similar, cyanogenic b-glucosidase. Based on the crystal structure of a plant myrosinase with bound substrate identified by the HMM, the residue F354 is likely to be on the surface of the Klotho1 domain and have no direct role in catalysis. However, the residue corresponding to C372 is interspersed between the nucleophile and proton donor, and thus may influence Klotho function by affecting the activities of the catalytic residues or modifying the substrate binding and/or recognition properties of Klotho1. These data suggest that variation in Klotho function contributes to heterogeneity in the onset and severity of human age-related phenotypes.

Linkage Disequilibrium mapping of quantitative trait loci: multiple trait analysis. *J.M. Akey, L. Jin, M. Xiong.*
Human Genetics Center, University of Texas, Houston, TX.

Many phenotypes of medical and economical interest show quantitative variation in the population. The broad purview of quantitative traits is further evident by recognizing that even qualitative diseases, such as the presence or absence of essential hypertension, result from underlying quantitative variables. Although it is widely recognized that identifying individual quantitative trait loci (QTL) would be of profound practical importance, little progress in this area has been made. Recently, the proposition of constructing a dense single nucleotide polymorphism (SNP) marker map has stimulated considerable interest in Linkage Disequilibrium (LD) mapping of complex trait loci. Most LD mapping methods proposed to date are based on one-locus one-trait models. However, genes do not exist as isolated systems and it is likely that one QTL will have multiple phenotypic affects. Therefore, the joint analysis of multiple quantitative traits may improve the power of QTL mapping as well as provide fundamental insights into their genetic architecture. To this end, we present a novel regression approach for LD-based multiple trait analysis (MTA) of a QTL that simultaneously affects several quantitative traits. Specifically we will: 1) introduce a statistical framework for LD-based MTA, 2) provide a formal set of procedures and hypotheses concerning both the existence of a QTL and the genetic correlation structure of multiple quantitative traits, 3) present analytical formula for calculating the power of LD-based MTA, and 4) demonstrate how the power of LD-based MTA is influenced by important parameters such as the genetic distance between marker and QTL, heritability explained by the trait locus, residual correlation between traits, dominance deviation, and strength of initial LD. In general, we conclude that MTA can significantly improve the power of mapping a QTL, with the gain of power dictated by the magnitude and direction of the residual correlation between traits. Finally, we will demonstrate the utility of LD-based MTA by applying this method to systolic and diastolic blood pressure in an isolated Chinese population.

Number of QTLs and their effects on plasma HDL levels in familial combined hyperlipidemia. *F. Gagnon, G.P. Jarvik, A.G. Motulsky, S.S. Deeb, J.D. Brunzell, E.M. Wijsman.* Department of Medicine, Univ. of Washington, Seattle, WA.

Familial combined hyperlipidemia (FCHL) is defined by elevated levels of plasma cholesterol and/or triglyceride. Several modifier genes have been reported i.e. genes having a modifying effect on FCHL associated phenotypic traits, such as plasma high-density lipoprotein cholesterol levels (HDL). However, major genes are as yet unknown. Evidence for linkage in FCHL has been reported in some studies, but without confirmation in other populations. The inability to detect strong linkage and/or to confirm reported linkage, coupled with recent segregation analysis results, suggests that FCHL is heterogeneous. Our ability to localize genes depends on the number of contributing loci and the contribution of each to the phenotype. The purpose of our study was to estimate the number of quantitative trait loci (QTLs) involved in the regulation of HDL in FCHL pedigrees, and to estimate their individual effects. A novel analytic strategy based on Monte Carlo Markov chain methods allows us to estimate the number of contributing loci and their contribution to variance in HDL. We performed an oligogenic segregation analysis of HDL as a quantitative trait using 183 individuals in 3 FCHL pedigrees. Genetic effects account for ~ 59% of the variance in HDL, with environment and sex contributing ~ 33% and 8%, to the variance, respectively. We estimate a mean of 2.3 QTLs contributing to HDL levels. The largest QTL accounts for more than 71% of the total genetic variance. This QTL is a dominant/recessive locus. The frequency of the common allele at this locus is estimate to be 0.6. With the common homozygote genotype fixed as reference value, the heterozygote has an estimated effect of 7.6 mg/dl (13.7 SD) and that of the less frequent homozygote is 21.7 mg/dl (28.3 SD). The QTL with the second largest effect is responsible for ~ 22% of the genetic variance. The remaining QTLs together account for less than 7% of the genetic variance. Due to the magnitude of its effect, the largest QTL is promising for gene localization. A genomic search, also exploring the genetic influence on HDL subfractions, HDL₂ and HDL₃, is under way.

Incorrect inferences of heterogeneity for genes with common susceptibility alleles. *J.A. Badner.* Dept Psychiatry, Univ Chicago, Chicago, IL.

It has been shown that the heterogeneity lod score (HLOD) can be at least as powerful as non-parametric tests for the detection of linkage in complex genetic traits when the correct mode of inheritance is modeled. What is less clear is the relationship of the parameters modeled or estimated (allele frequency, genetic model, proportion of families linked) to the actual parameters of the susceptibility gene. For example, given a common gene for which all affected individuals carry the susceptibility allele, a substantial number of families will not give evidence of linkage due to homozygosity in the parents. Thus, there may be the appearance of genetic heterogeneity when in fact there is not. To test this, simulations were performed of a genetic model with 5 epistatic genes with common susceptibility alleles leading to an incidence of the trait of ~1% and a total relative risk to sibs of ~10. Families with 3 sibs were simulated and families were selected for linkage analysis if at least 2 of the sibs were affected. Linkage analysis was performed with Genhunter/PLUS (GHP) and dominant and recessive HLODs with allele frequencies lower than those simulated. Linkage analysis was performed on 100 or 200 families. Results demonstrate that the power of HLOD was comparable to GHP for the detection of linkage but that estimates of the proportion of families carrying the susceptibility was much lower than that simulated. There was wide variation in lod scores for all 3 analytic methods probably due to varying proportions of informative families. This would suggest that different studies with similar numbers of families could give conflicting results for linkage despite the lack of genetic heterogeneity.

An interleukin-1 genotype is associated with outcome of meningococcal disease. *N.J. Camp^{1,3}, R.C. Read¹, F.S. diGiovine¹, R. Barrow², E.B. Kaczmarek², A.G.A. Chaudhary¹, A.J. Fox², G.W. Duff¹*. 1) Division of Molecular and Genetic Medicine, University of Sheffield, U.K; 2) Meningococcal Reference Unit, Manchester Public Health Laboratory, U.K; 3) Genetic Research, LDS Hospital, IHC, Salt Lake City, UT.

Meningococcal disease has a mortality of 5-20% despite antimicrobial therapy and intensive care. The host's cytokine response reflects the severity of their disease (van Deuren et al., 1995, Westendorp et al., 1995) and is genetically determined (Westendorp et al., 1997). The cytokine IL-1 is a key mediator of the inflammatory response. There are three known IL-1 genes on human chromosome 2q13 (Nicklin et al., 1994); IL-1A and IL-1B which both encode for pro-inflammatory proteins, and IL-1RN which encodes the IL-1 receptor antagonist.

To test whether polymorphisms within pro-inflammatory cytokine genes are related to the outcome of meningococcal disease, we compared IL-1 genotype distributions in survivors and in fatal cases. Samples were included in the study if meningococcal disease was verified either by culture or PCR detection of *Neisseria meningitidis* in blood or CSF.

A significant association ($p=0.0005$) was found between fatal outcome and genotype at IL-1B(-511). Homozygous individuals, both 1/1 and 2/2, had increased relative risks for death compared to heterozygotes, 3.39 [1.39-8.29] and 7.35 [2.51-21.45] respectively. The percentages of individuals by genotype at IL-1B(-511) who died, were 18.0% (1/1), 6.1% (1/2) and 32.3% (2/2), compared to 14.2% overall. The composite genotype consisting of heterozygosity at IL-1B(-511) together with homozygosity for the common allele (1/1) at IL-1RN(+2018) was significantly associated with survival ($p=0.018$; OR = 7.78 [1.05,59.05]).

In conclusion, we propose that the genetic programming of the inflammatory response at the IL-1 locus influences clinical outcome in human *Neisseria meningitidis* infection.

Effects of difference in allele frequency between marker and disease variant and linkage disequilibrium between them on the power of genomewide association studies of complex disease genes. *J. Ohashi, K. Tokunaga.* Dept Human Gen, Grad Sch Med, Univ Tokyo, Tokyo, Japan.

In indirect genomewide association studies of complex diseases using a dense map of single nucleotide polymorphism (SNP) markers, whether a predisposing gene to the onset of the disease can be detected is dependent not only on the degree of linkage disequilibrium between the SNP marker and the disease variant but also on the difference in their allele frequencies. In order to investigate influences of these factors on the statistical power, we calculated the required number of samples, for the detection with a 80 % power, in population-based association studies. The result showed that a large difference between disease and marker allele frequencies does not allow us to detect an association of the marker with the disease, even if the marker is in complete linkage disequilibrium with the disease variant. In general, disease variants are rare and in positive linkage disequilibrium with major SNP alleles rather than minor ones. Thus, a remarkable reduction in statistical power is unavoidable in the genomewide screening of complex disease genes due to the difference in allele frequency. If a large number of markers are tested, the problem seems to be solved. However, the obtained p-value may not reach significance level due to the Bonferroni adjustment. In addition, SNP alleles suitable for use as genetic markers were found to be very rare, based on the theory of population genetics. These results suggest that indirect genomewide association studies may not detect disease variants contributing modestly to the onset of a complex disease even if a large number of SNP markers are available.

Association of blood pressure and polymorphisms in the promotor region of catalase in a Chinese population. Z. Jiang^{1,2}, J. Shi², J.M. Akey³, M.M. Xiong³, Y. Wang², Y. Shen², X. Xu^{1,2}, H. Chen^{1,2}, H. Wu², J. Xiao², D. Lu¹, W. Huang², L. Jin^{1,2,3}. 1) Institute of Genetics, Fudan University, Shanghai, Ch, Shanghai, China; 2) Chinese National Human Genome Center at Shanghai, China; 3) Human Genetics Center, University of Texas, Houston, Texas.

Essential Hypertension (EHYT) is a complex multifactorial disease, which represents a major burden to public health. A useful conceptual model regarding the etiology of EHYT is that it results from the interaction of several deleterious intermediate phenotypes, which may be under distinct genetic control. An attractive intermediate phenotype that may be involved in the pathogenesis of EHYT is the system of antioxidant genes that acts to minimize the potential harmful effects of reactive oxygen species (ROS). An integral member of the antioxidant system is catalase, which detoxifies hydrogen peroxide (H₂O₂) into water and molecular oxygen. To address the role of catalase in EHYT we ascertained 300 unrelated individuals from an isolated Chinese population and screened those who fell in the top and bottom 10% of the blood pressure distribution for genetic variation. Three single nucleotide polymorphisms (SNPs) were found in the promoter region. We used a novel and powerful statistical method, linkage disequilibrium based regression (LDR), and a unique study design to test for association of the identified SNPs with EHYT. Specifically, we performed LDR on individuals whose systolic blood pressure (SBP) was greater than a selected threshold (i.e., SBP³150 and SBP³160). A C/T SNP 869 base pairs (SNP869) upstream of the start codon was found to be significantly associated with EHYT. As the selected threshold increases the p-values decrease (SBP³150, p=0.0083; SBP³160 p=0.0020). Interestingly, SNP869 lies in a putative recognition site for several important tissue specific transcription factors: GATA, LMO-2, OCT-1, and MZF1. To our knowledge this is the first study to implicate genetic variation in catalase with EHYT.

Hardy-Weinberg disequilibrium (HWD) fine mapping for case-control samples. *D.V. Zaykin¹, D.M. Nielsen²*. 1) US Bioinformatics, GlaxoWellcome Inc., Res Triangle Pk, NC; 2) Department of Statistics, North Carolina State University, Raleigh, NC.

Nielsen et al. (1998) showed how HWD can be used to make inferences about location of disease genes in samples of affected individuals (cases). A potential difficulty in applying the method to actual data is uncertainty whether the observed HWD is caused by linkage disequilibrium with the disease gene, or by other population-genetic phenomena, such as population stratification. We demonstrate that when samples of non-affected individuals (controls) are available, the difference in HWD between cases and controls can be used to alleviate the problem. We propose a statistical procedure to estimate significance of that difference. Further, we study type I error rate and statistical power of the new method in samples from simulated homogeneous and admixed populations. We find that under the hypothesis of no linkage disequilibrium between the disease gene and a marker, the probability of rejection for the usual case-control contingency test is independent of that for the new test. Thus, the test we propose conveys independent evidence in marker-disease association studies.

Association of MSX1 with Nonsyndromic Orofacial Clefting in ECLAMC. *A.R Vieira^{1,2,5}, I.M. Orioli², E.E.*

Castilla³, S.E. O'Brien¹, M.L. Marazita⁴, M.E. Cooper⁴, J.C. Murray¹. 1) Dept. Pediatrics, Univ. Iowa, Iowa City, IA; 2) Dept. Genetics, UFRJ, Brazil; 3) Dept. Genetics, FIOCRUZ, Brazil & CONICET, Argentina; 4) Cleft Palate Center, Univ. Pittsburgh, PA; 5) Bolsista da CAPES, Brazil.

ECLAMC (Latin American Collaborative Study of Congenital Malformations) is a program which investigates the cause of congenital malformations and their frequencies in Latin American hospitals. Since January 1998, ECLAMC has been collecting biological samples from children with nonsyndromic orofacial clefts (NSOFC) and their mothers for molecular analysis. NSOFC are common congenital malformations known to occur in approximately 1/1,000 live births in ECLAMC hospitals. However higher frequencies of NSOFC have previously been reported in subsets of the South American population, such as Amerindians and cases from higher altitudes (2,000 or more meters above sea level). Past studies suggested multifactorial causation for NSOFC, having both genetic and environmental origins. MSX1 has been associated with craniofacial anomalies in mice as well as clefting and/or hypodontia in humans. The present study uses linkage disequilibrium (LD)-based analysis to detect non-Mendelian transmission of MSX1 alleles in a total of 171 South American children and their respective mothers. To date, we have completed the genotyping for MSX1 using a CA-repeat marker. The results show LD between NS cleft lip with/without cleft palate ($p < 0.0001$; $n = 152$) and for cleft palate only ($p < 0.05$; $n = 19$) both with allele 4. This supports previous linkage and LD findings with MSX1 in humans and suggests MSX1 mutations are a substantial contribution to clefts in South American populations. Further investigations will use direct sequencing and population-specific candidate gene disequilibrium for NSOFC. To do this an admixture approach is being used to estimate the frequency of Amerindian-specific mitochondrial haplotypes and Y chromosome markers in NSOFC probands. If a significant overrepresentation between these markers and NSOFC is found, this would suggest an increase for admixed Amerindian-specific genes in the incidence of NSOFC. This might allow practical genomewide disequilibrium mapping to then be carried out.

Association of variation in the promoter of the Beta-2 Adrenergic Receptor and essential hypertension in an isolated Chinese population. *H. Chen¹, J.M. Akey², M.M. Xiong¹, H. Xu¹, J. Xiao², L. Jin^{1,2}.* 1) Institute of Genetics, Fudan University, Shanghai, China; 2) Human Genetics Center, University of Texas, Houston, Texas.

Essential Hypertension (EHYT) is a quintessential complex disease, whose phenotype is the culmination of multiple genetic and environmental factors. In considering the etiology of EHYT it is instructive to consider the interaction of intermediate pathways in disease pathogenesis. The sympathetic nervous system is an important pathway in blood pressure regulation by controlling diverse processes such as vascular responsiveness, cardiac output, and renal sodium handling. A key component of this pathway is the beta-2 adrenergic receptor (BAR2), a G-protein coupled receptor, that is primarily expressed in skeletal and cardiac muscle cells. To address the role of BAR2 in the pathogenesis of EHYT we ascertained 300 unrelated individuals from an isolated Chinese population and screened those who fell in the top and bottom 10% of the blood pressure distribution for genetic variation. In total we identified 7 SNPs throughout the coding and non-coding regions of BAR2, all of which have been previously described. We used a novel and powerful statistical method, linkage disequilibrium based regression (LDR), and a unique study design to test for association of the identified SNPs with EHYT. Specifically, we performed LDR on individuals whose systolic blood pressure (SBP) was greater than an artificially selected threshold (i.e., SBP³150). Of the 7 SNPs identified we found that a G/A SNP 1,023 base pairs upstream of the start codon (SNP1023) was significantly associated with EHYT ($p=0.0046$). *In silico* promoter analysis revealed SNP1023 lies 2 bp 5' to the consensus sequence of MyoD (referred to as the E-box), an important bHLH transcription factor regulating differentiation of muscle tissue. Interestingly, previous studies have demonstrated that flanking base pairs of the E-box can mediate the efficiency of transcription factor binding. To our knowledge this is the first study to implicate SNP1023 of BAR2 with EHYT.

Gene-environment interaction in complex traits: A study of the Korean diaspora. *J.D. Terwilliger, A. Rzhetsky, J.H. Lee.* Columbia University, NYC, NY.

In researching the etiological basis of multifactorial traits, geneticists tend to overstate the importance of marginal effects of single genetic factors (integrating out the environment), while epidemiologists overstate the importance of individual environmental factors (integrating out genetics), while the truth is likely that the action is in their interaction, and one would be better off with a study designed to test both genes and environment jointly. Epidemiologists sometimes control for genetics through the use of migrant studies. For example, they might compare Koreans living in Japan with Koreans living in Korea, and Japanese living in Japan, to estimate the relative impact of genetic and environmental risk factors (results of which are a function of the degree of acculturation of the migrants). Unrelated individuals are normally studied to identify environmental risk factors, controlling for confounding effects of genes. However, a family-based study of diasporic populations allows one to examine interactions between genes and environment, comparing familiarity in different environmental strata - which is especially powerful when such strata are extremely divergent.

We focus on populations of the Korean diaspora, living in very different environmental and cultural contexts. Koreans have the 5th largest diasporic population in the world, most emigrating after 1860, with low rates of exogamy. There are about 2,000,000 Koreans living in China and the USA, 500,000 in the former Soviet Union and Japan, and many more in S. America and Australia. The Koreans of Kazakhstan are of special interest. They are extremely acculturated as the result of their forced migration in 1937, and subsequent suppression of their culture. Those in the USA emigrated mostly after 1965, and have quite different lifestyle from those in Korea. We compare these groups to families from the ROK. Additional populations of future interest include thousands of adoptees living in Northern Europe, raised by European families, and the Korean populations of Manchuria and Japan. Our study focuses on quantitative risk factors for multifactorial disease, and the importance of interactions between genetic and environmental risk factors in their etiology.

A strategy to analyze linkage heterogeneity and to detect gene-gene interaction with a model-free approach applied to bipolar disorder. *E. Shink, J. Morissette, V. Raymond, N. Barden.* Neuroscience, CHUL, Ste-Foy, Quebec, Canada.

Objective: Bipolar affective disorder is a complex disease where the phenotype may result from the epistatic interaction of several loci. We have completed a genome wide scan on 20 families of different size from the Saguenay-Lac-St-Jean region of Eastern Quebec and found definitive evidence of linkage between bipolar affective disorder and a locus situated in the 12q23-q24 region. We tried to find out if gene-gene interaction occurred between this region and other regions where weak evidences of linkage were found or between these other areas of linkage. Method: Chromosomal regions, where LOD scores over 1 were observed for 2 successive markers using parametric or model-free approaches, were selected for interaction analysis. For each marker within the selected region, we estimated with ASPEX, the proportion of alleles shared IBD by affected sib-pairs. For each region the proportion of shared alleles was used as variable for a Principal Component Analysis and the first axis as an index of linkage. Correlation analysis was done on these indexes to detect heterogeneity or epistasis. Fisher algorithm was used to classify families into two groups as linked or unlinked to a particular locus. Results: A negative correlation was observed between the chromosome 12 region of definitive linkage and the chromosome 15 area ($r=-0.51$; $p=0.023$). On the other hand, positive correlations were obtained between regions of chromosome 15 and chromosome 2 ($r=0.60$, $p=0.005$) and between regions of chromosome 2 and chromosome 17 ($r=0.65$, $p=0.002$). Cluster analysis (Fisher) suggested that 11 families out of 20 were linked to chromosome 12. The group obtained for chromosome 15 and considered as linked families produced a cumulative LOD score of 3.16. Conclusion: We have used a model-free approach to study linkage heterogeneity and gene-gene interaction and applied this to a sample of families with bipolar affective disorder. Results suggest that there is no epistasis between a confirmed susceptibility locus on chromosome 12 and any other locus. However, evidence for heterogeneity with a susceptibility locus situated on chromosome 15 was found.

Reproductive pathology and offspring outcomes in women with schizophrenia and affective psychoses: genetic vulnerability or environmental risk? A.V. Jablensky¹, S.R. Zubrick², C. Bower², V. Morgan¹, L.A. Yellachich¹, T. Pinder³, P. Montgomery⁴. 1) Department of Psychiatry, University of Western Australia; 2) Institute for Child Health Research, Perth; 3) Health Department of Western Australia; 4) Disability Services Commission, Western Australia.

While a number of studies have examined pregnancy and birth abnormalities in mothers of adults with schizophrenia, limited evidence is available on pregnancies and offspring outcomes in women with schizophrenia or affective psychoses. The present study, based on record linkage across 5 prospectively cumulated population databases (psychiatric case register; maternal and child health database; birth defects registry; cerebral palsy register; and intellectual disability register) identified 1831 women born 1931 - 1980 who had been diagnosed with schizophrenia (N=382) or affective psychosis (N=1449) and given birth to a total of 3174 children. A random sample of 1831 women without psychiatric diagnoses, with a total of 3129 children, was chosen as a control group. Both schizophrenic and affective cases differed significantly from controls in having increased risks of: overall pregnancy, birth and neonatal complications as measured by the McNeil-Sjostrom scale; placenta praevia (OR 2.06); antepartum haemorrhage (OR 1.61); and foetal distress (OR 1.25). Women with schizophrenia were more likely to have placental abruption (OR 3.45), precipitous delivery (OR 1.57), postpartum haemorrhage (OR 1.66), and to give birth to infants in the lower weight/growth and head circumference centiles when controlled for gestational age, sex, maternal height and parity. The offspring of mothers with schizophrenia was at a substantially elevated risk of death in infancy or early childhood (OR 4.1). There was a highly increased risk of mental retardation in the offspring of both women with schizophrenia (OR 6.9) and women with affective psychoses (OR 4.6); and of epilepsy in the offspring of women with affective psychoses (OR 5.4). While interaction between genetic vulnerability and environmental factors is likely to account for some of these effects, significant risk reduction may be achieved through psychosocial and behavioral intervention.

The contribution of genes and prenatal environment to insulin resistance. *R. Vlietinck, R. Loos, R. Fagard, G. Beunen, C. Derom.* Katholieke Universiteit Leuven, Leuven, Belgium.

Twins have been used to estimate the genetic component in chronic diseases in the general population. The classical twin study is based on the assumption that monozygotic twins (MZ) are genetically identical, whereas dizygotic twins (DZ) share on average half of their genes. The different prenatal environment of monozygotic monochorial twins (MZ MC) compared to the monozygotic dichorial (MZ DC) is never taken into account. However, monochorial placentation allows exchange of blood, hormones, enzymes, etc... The fetal origins hypothesis predicts MZ MC might experience a higher concordance of chronic disease, due to a more similar prenatal environment. In the present study we investigated whether the prenatal environment contributed to the variance of insulin resistance, besides the influence of genes and 'postnatal' environment. Twins (112 DZ, 88 MZ DC, 111 MZ MC, 25.7±4.8 yr) were selected at random from the East Flanders Prospective Twins Survey. Zygosity was determined through blood groups and DNA fingerprints, reaching a probability of 0.95 or more. Fasting plasma insulin and glucose concentrations were determined to assess insulin resistance with the homeostasis model assessment (HOMA). Comparing the intra-pair correlation of MZ and DZ twins suggests the presence of a genetic component (males: MZ .45 vs DZ -.03, females: MZ .51 vs DZ .20). However, when the MZ correlation is split up, there is some evidence for a prenatal factor in males (MZ DC 0.38 vs MZ MC .56) but less in females (MZ DC 0.57 vs MZ MC 0.47). We adapted the classical genetic model fitting by including a 'prenatal environment' factor. The model that fitted the data best included a genetic factor, a prenatal environmental factor and a unique environmental factor, accounting for respectively 32%, 25% and 43% of the variance of insulin resistance in males and for 44%, 12% and 44% in females. Since there is an increasing body of evidence that chronic diseases may partly originate from prenatal experiences, we suggest adapting the classical twin study by including a 'prenatal factor'. In addition, this allows evaluating the relative importance of genes, prenatal and postnatal environment.

Molecular diagnosis and cascade screening modify the epidemiology of hereditary haemochromatosis. V. Scotet¹, M.C. Merour², A.Y. Mercier², B. Chanu², T. Le Faou², O. Raguene¹, G. Le Gac², C. Mura¹, C. Ferec^{1,2}. 1) Dept Molecular Genetics, Hospital, University, Brest, France; 2) EFS-Bretagne, France.

Hereditary haemochromatosis (HHC) is the most common inherited disorder in populations of Northern Europe. The disease, characterised by an iron overload, is treated by regular venesections but remains short-term prognosis without treatment. The gene responsible for HHC (HFE gene) was identified in 1996 and three mutations have been reported: the C282Y, found in 70 to 95% of patients, the H63D and the S65C associated with a milder form of HHC. The identification of this gene has enabled to implement efficient strategies of diagnosis and prevention, notably cascade screening, which allows to detect homozygous subjects in pre-symptomatic phase and therefore to avoid severe forms of HHC. The aim of this study was to assess the impact of molecular diagnosis on the epidemiology of the disease from a cohort of 373 subjects homozygous for the C282Y followed in a blood center of western Brittany, France. In this study, 62.7% of the subjects were males. We noticed a significant decrease of the sex-ratio, which was 3.71 (126/34) before the discovery of the gene, and 1.03 (108/105) following the development of genetic test ($p < 10^{-6}$). The circumstances of HHC diagnosis also evolved; the proportion of subjects detected by family testing greatly increased following the introduction of molecular testing, especially in females (from 32.3% to 47.1%), in whom the age at onset tended to decrease (from 51.9 y. to 48.2 y.). Moreover, the diagnosis is now more frequently made following a biological check-up in subjects who complain of fatigue. Finally, the symptoms associated at the time of onset are less frequent, particularly skin pigmentation (26.1% versus 38.8% - $p = 0.012$) and hepatomegaly (10.2% versus 23.7% - $p < 0.001$). This study highlights the importance of molecular diagnosis of HHC which enables, through family testing, earlier identification and treatment of patients, mainly of females. The pre-symptomatic diagnosis, which leads to a modification of HHC epidemiology, and the preventive follow-up should be crucial for patients to preserve a normal life expectancy.

Relative power of case-control and simple random sampling association studies of candidate gene variants. *W.H. Kao, F.L. Brancati, T. Beaty, J. Coresh.* Dept Epidemiology, Johns Hopkins Univ/Sch HPH, Baltimore, MD.

With rapid advances in human genetics, more association studies of candidate gene variants or single nucleotide polymorphisms (SNPs) will be conducted on complex traits, but little is known about the relationship between study designs and power. We investigated these questions as they apply to studies of obesity and its candidate variants: 1) Is a case-control (CC) study of a dichotomized quantitative trait that compares genotypic frequencies between obese cases and lean controls more powerful and cost effective than a study of a simple random sample (SRS) that compares mean body-mass index (BMI) across genotypes? 2) Does quantitative trait analysis (QTA) in a CC study increase its statistical power and type I error rate? Assuming sample size is 600 and BMI for the unaffected genotype $\sim N(m, s^2)$, power for the two designs (CC and SRS) were evaluated under various heritabilities (h^2), prevalence of affected genotypes (P), and critical values defining controls (Ccn, controls have BMI < Ccn) and cases (Ccs, cases have BMI > Ccs]. For example, when $h^2=1\%$ and $P=0.2$, powers of CC (Ccn=Ccs=m), CC (Ccn=m, Ccs=m+s), CC (Ccn=m, Ccs=m+2s), CC (Ccn=m-s, Ccs=m+s) and SRS were 50%, 82%, 98%, 96% and 69%, respectively. In summary: 1) for a given sample size, the power of SRS was strictly a function of h^2 , 2) for any h^2 , a greater separation between case-control critical values led to higher power in CC, 3) CC was more powerful than SRS given that $Ccn < Ccs$ and $Ccs > m+s$, and 4) the cost effectiveness of the CC design relative to SRS depends on the cost ratio of genotyping:phenotyping. If this cost ratio for an individual is 1:1, the relative study cost of CC to SRS was approximately 1 for any h^2 . However, if this ratio were 10:1 or 100:1, as might be the case with genome mapping using numerous SNPs, the CC design was more cost efficient than SRS for any h^2 . In a simulation of 1000 CC studies ($N=600$), QTA did not result in higher type I error rates and yielded small gains in power. Thus, under many circumstances, a case-control design is more powerful and cost-effective than a study of a simple random sample for associations between quantitative traits and candidate variants.

Analysis of Buccal Swab DNA under Various Conditions. *K.S. Mann, H.M. Patterson, C.B. Sutcliffe, J.L. Haines.*
Program in Human Genetics, Vanderbilt University Medical Center, Nashville, TN.

Buccal swabs are an effective means of collecting DNA in a noninvasive manner. However, the quality of the DNA extracted from buccal swabs may be affected by such factors as storage conditions or immediacy of extraction. Previous studies conducted in this laboratory showed immediate extraction of DNA from buccal swabs using the QIAamp Blood Mini Kit(Qiagen™) yielded efficiently amplifying DNA. Further experiments were performed to define factors affecting DNA yield. Firstly, the effect of temperature storage conditions on buccal swabs and subsequent DNA was addressed. Secondly, the relative stabilities of DNA extracted from blood and buccal swabs were compared. Thirdly, short tandem repeat(STR) loci were amplified from buccal or blood derived DNA to assay for mosaicism.

Ten buccal swabs were collected from each of 20 individuals using a Cytosoft™ brush(Medical Packaging Corp.). In experiment 1, four swabs from each individual were stored for variable time periods under differing temperature conditions before being extracted and quantitated. Storage times ranged from 0 to 48 hours, and storage temperature was either 4°C or room temperature.

Six of the 10 swabs from each individual were immediately extracted using the QIAamp kit and quantitated for use in experiments 2 and 3. The amount of DNA in these samples ranged from 1ng to 45ng. Five of the 20 individuals donated a 7.5ml blood sample, which was extracted using a Puregene® Kit(Gentra Systems) and quantitated for use in experiments 2 and 3. Blood DNA yields ranged from 220mg to 303mg. For the DNA stability and mosaicism experiments, highly informative STR markers were used. Also, 6 of the 6 samples that had been extracted >12 months ago and stored at 4°C were successfully requantitated and amplified. DNA amounts per sample ranged from 7ng to 45ng.

DNAs were amplified with Cy-3 labeled Amelogenin A and B primers as well as various STR markers. Fluorometry was used to quantitate all samples. Samples were run out on 6% denaturing polyacrylamide gels.

Maternal and Paternal Affinities in Mediterranean Populations. *N. Frenkel-Arons¹, M. Korostishevsky¹, Y. Pel-Or¹, A. Berry¹, E. Woolf², Y. Hillel², B. Bonne-Tamir¹.* 1) Dept. of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel-Aviv University, Ramat-Aviv, Israel 69978; 2) Hebrew University Faculty of Agriculture, Rehovot, Israel 76100.

Mitochondrial DNA HVI and HVII control regions were sequenced in 266 individuals including Moroccan, Libyan, Turkish and Bulgarian Jews, Druze and Palestinians. Our results were compared with existing data on Turkish and Bulgarian non-Jewish populations. Maximum diversity in the control region segments was found in Turkish non-Jews and in Palestinians. Nucleotide differences within these two populations was higher than between either of them and any other ethnic group in the study. The lowest diversity within groups was observed among Moroccan Jews. Largest genetic distances based on nucleotide difference were revealed between Libyan Jews and all other Jewish groups.

Six microsatellite markers of Y-chromosome were typed in the males of the same four communities (Moroccan, Libyan, Turkish, and Bulgarian Jews). In contrast with the mitochondrial results, Libyan Jews showed substantial similarity to Y-chromosome haplotypes of Moroccan Jews, while Turkish Jews demonstrated even higher affinity to Bulgarian Jews.

The difference between mitochondrial and Y-chromosome results suggests that females married into the populations at a much higher rate than males did. This divergence between maternal and paternal gene flow has been observed in other world populations and has implications for tracing the particular histories of various ethnic groups.

A comparison of autosomal, mtDNA and Y-chromosome diversity in Native South Americans. *N. Mesa¹, M.C. Mondragon¹, I.D. Soto¹, M.V. Parra¹, C. Duque¹, D. Ortiz-Barrientos¹, J.G. Munera¹, L.F. Garcia¹, I.D. Velez¹, G. Bedoya¹, M.C. Bortolini^{2,3}, A. Ruiz-Linares^{1,3}.* 1) Facultad de Medicina, Universidad de Antioquia, A.A. 1226 Medellin, Colombia; 2) Departamento de Genetica, UFRGS, Porto Alegre, Brazil; 3) Department of Medical and Community Genetics, Imperial College School of Medicine, U.K.

To evaluate the proposed higher migration rate of females throughout human evolution (Nat. Genet. 20: 270, 1998) we examined the level of within and between population diversity in 5 Colombian Native American populations. Markers examined included: mtDNA (5 RFLPs), autosomal (11 microsatellites, 5 beta globin gene cluster RFLPs and the MHC DQA1 locus) and Y chromosome polymorphisms (7 microsatellites and 4 biallelic polymorphisms). The table below summarizes estimated G_{st} and N_m values for the markers typed. A mean G_{st} of 6.8% was observed for autosomes, a figure in agreement with larger marker/populations surveys. Consistent with a smaller N_e, both mtDNA and Y-markers show higher levels of diversification than autosomal markers. However estimates of N_m are very similar for all sets of markers. Rates of admixture vary between the populations examined but these do not seem to seriously affect our estimates. In conclusion, we found no evidence for a higher migration rate of females in the populations examined.

	mtDNA	Y-STR	Y-SNP	Beta Globin RFLPs	DQA1	Autosomal STR
G _{st}	0.180	0.165	0.269	0.089	0.116	0.061
N _m	4.5	5.1	2.7	2.6	1.9	3.8
Mean G _{st} Y-markers	0.184	Mean G _{st} Autosomal markers	0.068			
Mean N _m Y markers	4.4	Mean N _m Autosomal markers	3.4			

Mutations in the CX26 gene in the Netherlands. *L.H. Hoefsloot, M. Siers, M. Geurds, E.C.M. Mariman.* Dept. Human Genetics, UMC-St. Radboud, Nijmegen, The Netherlands.

There are at least 40 different genes involved in both autosomal dominant and autosomal recessive non-syndromal deafness, but mutations in the connexin 26 gene (*CX26*) form a major cause. In addition, mutations in *CX26* have been described in syndromal deafness (Vohwinkels' Syndrome). In the context of routine DNA-diagnosis, the *CX26* open reading frame was screened for mutations. In 80 unrelated persons with non-syndromal deafness both from affected sibships and sporadic patients, no mutation was found in 57 (71%). In 17 patients two mutations were found (21%), and in 6 (8%) only one mutation was detected. In total we found 41 mutant alleles, 26 (63%) carrying the common 35delG mutation. The other mutations were found only once or twice, and some have been described before. Two new deletions and a new insertion were found, and four new missense mutations. Two of the missense mutations were found as the only mutation, indicating that it concerns either a dominant form of deafness, or that they are rare alleles, because they were not found in 50 control persons. These results are in accordance with the findings of others, and confirm that in cases of non-syndromal deafness, screening *CX26* for mutations is worthwhile.

Clinical trial sample sizes needed to confirm a genotype-adverse drug reaction association. *M. Mosteller.* Division of Clinical Genetics, Glaxo Wellcome, Inc, Res Triangle Pk, NC.

Pharmaceutical companies are actively searching for genetic markers that predict increased risk of adverse drug reaction (ADR). Initial indication of a genotype-ADR association may come to light through a retrospective analysis of clinical trial data using markers within candidate genes, although evaluation of a SNP (single nucleotide polymorphism) marker set covering the entire genome is rapidly becoming a possibility. In any event, for such genotype-ADR associations to be practically applied, additional clinical trial data will have to be analyzed to confirm initial findings and to characterize the predictive power of a genetic "medicine response profile" that could be used in clinical practice. Simulation was used to explore the impact of genetic and non-genetic factors on required sample sizes for these analyses, under a set of assumed conditions: 1) a "causative" allele exists that enhances ADR risk in homozygous individuals, 2) a marker locus is in linkage disequilibrium (LD) with the causative locus, and 3) a matched-pair case-control design is used to confirm the predictive nature of the genetic marker. Clinical trial outcomes were simulated after specifying values for the following parameters: ADR rate among treated individuals, number of case-control pairs, allele frequencies at the marker and causative loci, degree of LD between marker and causative loci, penetrance of the risk-enhancing genotype, and phenocopy rate. Statistical power was estimated as the percentage of simulated trials in which the null hypothesis of no association between marker genotype and ADR occurrence was rejected. Sample requirements were evaluated for a variety of parameter value combinations. For example, if alleles at the marker and causative loci are equally frequent, LD is 75% of it's maximum possible value, and the phenocopy rate is 25% of the ADR rate, about 35 case-control pairs are required to achieve 80% power. Larger sample sizes are required if LD or penetrance is low, or if the phenocopy rate is high. Required sample sizes are unaffected by ADR rate. The matched-pair design requires fewer total subjects than a traditional retrospective case-control design.

A CYP2A6 promoter SNP which results in lower expression and is associated with asthma. *J.M. Hall¹, M. Guida¹, P. Hopkins¹, T. Williams¹, C. Davis¹, J. Witte².* 1) PPGx Inc, La Jolla, CA; 2) Case Western Reserve University Cleveland, Ohio.

The phenobarbital-inducible P450 gene, CYP2A6, a member a multigene family located on human chromosome 19 plays an important role in the steady-state levels of endogenous ligands involved in homeostasis, growth, differentiation, and neuroendocrine functions as well as in metabolizing environmental chemicals and drugs. Induction of CYP2A6 by phenobarbital is mediated almost entirely at the level of transcription. We identified 5 SNPs in 750 bp of the CYP2A6 promoter. One of them, a T to G transversion at position -39 from the start of transcription, is located within the putative CYP2A6 TATA box, changing the TATA sequence into TAGA, which seemed likely to affect CYP2A6 gene expression. To measure the transcriptional effect of this SNP, we subcloned a 744bp PCR product that contained either the T-39 or G-39 allele a luciferase expression vector. The level of luciferase activity obtained from the G-39 variant was 4 fold-less than that obtained from the T-39 construct. We determined the T-39 and G-39 allele frequencies in a collection of 1181 DNAs from 4 ethnic groups (Caucasian, African American, Hispanic and Asian Americans). The G-39 allele had different frequencies among these groups; the highest frequency was 23% in Asian Americans and the lowest frequency was 6% in Caucasians. Lack of CYP2A6 enzyme activity has been linked to nicotine addiction (Tyndale et al. *Nature*(98) 393:750). We found among 263 smokers those with the G-39 variant had slightly decreased cigarette consumption ($p=0.1$). There was no difference in variant status comparing smokers ($n=263$) to non-smokers ($n=426$). Since CYP2A6 is expressed in lungs and may be involved in metabolizing environmental factors that might trigger asthmatic reactions, we examined the association of variant status and asthma in a case-control study of 221 asthmatics and 261 non-asthmatics. Individuals with the G-39 allele have a 2-fold decreased risk of developing asthma than if they are homozygous for the T-39 allele ($p=0.04$). This suggests that the G-39 variant may protect against developing asthma.

Change in functional status over 2 years for Danish twins age 75+ using GoM scores to represent phenotype. *M.A. Mulder¹, K. Christensen², M.A. Woodbury¹, E.H. Corder¹*. 1) Center for Demographic Studies, Duke University, Durham, NC; 2) Health Sciences, The University of Southern Denmark, Odense, DK.

The extent to which health and function in old age is influenced by genetic factors is not well defined. We investigated the heritability of change in measures of basic functioning over two years for Danish twins age 75+. Specifically, 4494 person-year records from the 1995 and 1997 Longitudinal Study of Aging in Danish Twins were used to construct phenotype profiles by means of Grade-of-Membership (GoM) analysis. Information on each item in activities of daily living and instrumental activities of daily living inventories yielded five profiles of health and functioning. The heritability of membership in each phenotype profile was investigated using structural equation models. Each model included 223 pairs with information at time 1 and time 2 (91 MZ, 132 DZ). In an ACE model for profile 1 'Healthy', both additive and environmental components determined function ($a=-0.22$, $c=0.11$, $e=0.29$). Environment influenced short-term change in function ($e=0.27$), i.e. over two years. Health and function in old age is heritable in part. However, short-term change in health and function reflects individual environment.

Dopamine system genes and attention deficit hyperactivity disorder: evidence for gene-gene interaction. *B.S. Maher, M.M. Vanyukov, M.L. Marazita, H.F. Simkevitz, L. Kirisci, R.E. Ferrell, G.P. Kirillova, R.E. Tarter.* University of Pittsburgh, Pittsburgh, PA.

The dopamine system is hypothesized to play a major role in variation in the liability to Attention Deficit Hyperactivity Disorder (ADHD) and its underlying dimensions. We applied a measured-genotype approach to investigate the association of polymorphisms at several dopamine system genes with ADHD symptom count and indices of hyperactivity-impulsivity (HI) and inattention (In) in a population of 10-12 year old Caucasian males. The dopamine transporter (DAT1) and dopamine D2 (DRD2) receptor genes were significantly associated with ADHD symptom count and inattention indices. The dopamine receptor D4 gene was positively associated with ADHD symptom count. Importantly, we detected a significant interaction between genes for two dopamine receptor families, for ADHD symptom count and HI symptom indices (DRD2 & DRD5) as well as for inattention (DRD1 & DRD2).

MTHFR and MS polymorphisms are not related to hyperhomocysteinemia in Black populations. *C.E. Adjalla¹, E. Amouzou³, I. Abdelmouttaleb¹, C. Affo³, A. Sanni², J.L. Gueant¹*. 1) INSERM 00-14/Fac of Med Nancy-France; 2) Lab of Biochem Mol Biol-Benin; 3) Lab of Biochem and Nutr-Togo.

MTHFR and methionine synthase (MS) are 2 of the key enzymes in the folate/B12-dependent remethylation of homocysteine (Hcy) to methionine. Previous studies revealed that a polymorphism (PM) in the MTHFR gene C677T, causing a thermolabile enzyme, is related to hyperhomocysteinemia and coronary artery disease in the industrialized world. In these countries the prevalence of the homozygotes for the mutation was between 12 to 18% among Caucasians while this percentage was considerably low in Black descent (<2%). Another PM A1298C causing decreased enzyme activity and a silent mutation T1317C with a high allele frequency (39%) in a small group of Blacks have been identified. We described for the first time by using specific PCR-based RFLP method, frequencies of those 3 PM, and the A2756G PM in the MS in 240 subjects: 155 men, 85 women (31.7 \pm 9.3 years) from two West African countries. Hcy, folate and B12 levels were also assayed. Frequencies of the homozygotes for the C677T, A1298C, and T1317C were respectively 0.83%, 2% and 16%. The percentage of the homozygotes for the A1298C was lower than that already described (10-13%). This study confirmed the high frequency of the silent mutation T1317C in Blacks. The percentage of the homozygotes for the A2756G was 5.8%, this was consistent with those reported in other populations (4-5%). Subjects have a moderate hyperhomocysteinemia (18.0 \pm 1 mmol/L) according to the classification used for Caucasians. Folate was in the normal range (11.4 \pm 0.5 nmol/L) but we found a high B12 level (543.1 \pm 20.3 pmol/L). No correlation was found between the Hcy and either the C677T or the A1298C as described in Caucasians. The association of the 2 PM in the MTHFR was not related to the Hcy level. In conclusion, we showed a relatively high frequency of the silent T1317C in Black Africans compared to Caucasians in contrary to the 2 other PM (C677T, A1298C) in the MTHFR. The A2756G in the MS seems to be similarly distributed within Caucasians and Blacks. The PM in the MTHFR or MS are not related to moderate hyperhomocysteinemia in this subsaharan population.

New polymorphisms for COMT haplotypes can improve association tests for neuropsychiatric disorders. *M.M.C. DeMille, M.A. Palmatier, J.R. Kidd, V. Ruggeri, A.J. Pakstis, K.K. Kidd.* Genetics, Yale University, New Haven, CT.

An exon 4 SNP of the catechol-O-methyltransferase (COMT) gene on 22q causes a polymorphism in enzyme activity. In Palmatier et al. (1999, *Biol Psychiat* 46:557-567) we showed this functional variant, detected as an NlaIII RSP, is polymorphic in populations from all parts of the world. Two new polymorphisms in intron 1--a 67bp InDel ~3.9kb 5' from the NlaIII site and a tetranucleotide ~6.8kb 5' from the InDel--along with a previously known BglII RSP in exon 6, a silent substitution, have also been typed on 19 population samples to generate 4-site haplotypes. Heterozygosities range from .72 to .93 in these groups. Overall linkage disequilibrium (LD) for this 15kb interval, via permutation test, is strong and significant ($p < .001$ in 14 pops; $.001 < p < .006$ in 2 pops) in all but 3 populations: African Mbuti ($p = .06$), East Asian Ami ($p = .45$), and Micronesians ($p = .08$). Native American populations gave much stronger LD than populations in other world regions. 35 of 58 observed 4-site haplotypes (88 possible) occur at common frequencies ($>5\%$) in populations studied thus far allowing more refined COMT studies. Consistent with the known derived status of the low enzyme activity (LEA) allele at the NlaIII site, that allele occurs predominantly on 2 closely related haplotypes in these populations. (Though 10 of 35 haplotypes reaching common frequencies carry the LEA allele, 8 of these 10 are seen in just 1 to 5 groups.) In strong contrast, the ancestral high enzyme activity (HEA) allele occurs on 25 common haplotypes, 7 of which account for most of these chromosomes worldwide but none comes close to predominating like the main LEA haplotype. This complex pattern suggests additional functional variation may be missed by testing simply for association with the NlaIII site. The many studies trying to implicate functional differences in COMT enzyme activity with the etiology of neuropsychiatric disorders lack information about other sources of functional variation possibly arising elsewhere in COMT, such as the promoter region. Extended haplotypes could aid in identifying such variation. Supported in part by: GM57672, MH30929, NS01795, and SBR9632509.

DNA Polymorphism analysis in the Italian population and their use for paternity testing. *R. Galavotti, E. Trabetti, G. Malerba, P.F. Pignatti.* Sect Biol & Genetics, Dpt. Mother & Child, Biol & Gen, Univ. Verona, Italy.

We used several DNA polymorphisms for population genetic studies, linkage analysis, bone-marrow transplantation follow up, and parental testing. For every polymorphism we have determined allele frequency in the Italian population. The polymorphisms used were: D1S80, Apolipoprotein B (ApoB), FES/FPS, D12S1064 and D4S424 (Research Genetics), Amplitype HLA-DQA PCR Amplification and Typing Kit, Amplitype PM Kit (GYPA, GC, D7S5, HBGG, LDLR) and the AmpFISTR Profiler PCR Amplification Kit (D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820 and amelogenin), all kits from Perkin Elmer. Detection was by electrophoresis except reverse dot blot was used for the Amplitype kits. The allelic frequencies determined in our samples were similar to the available Caucasoid population data. The number of alleles detected varied from 5 for D12S1064 to 18 for D1S80 and ApoB, respectively. The observed heterozygote frequencies of the hypervariable loci ranged from 63% for TPOX to 90% for D3S1358. Genotype frequencies were in accord with Hardy-Weinberg equilibrium. For paternity testing a sequential protocol was used: 1) D1S80 and ApoB, 2) Profiler, 3) other polymorphisms, until either an exclusion with at least two different polymorphisms, or a probability of attribution of at least 99.99% was reached. Up to now we investigated 194 paternity cases, 108 of which were attributions and 86 were exclusions. The most informative polymorphisms in exclusions were D1S80 and ApoB with a capacity of exclusion of about 60% (59 exclusion/94 paternity cases). With the sequential protocol, the probability of attribution in the 86 exclusions varied from 0 (all the polymorphisms tested excluded) to 99.9% (4 polymorphisms excluded on a total of 13 tested). In conclusion, our experience indicates the importance of performing typing with enough markers (at least 11 in our protocol) in order to reach a probability of attribution of 99.99%.

Codon 129 genotyping in the prion protein gene as a diagnostic tool in Creutzfeldt-Jakob Disease. *E.A. Croes¹, S. Bulk¹, J.J. Houwing-Duistermaat¹, C.M. Vanduijn¹, W.A. Van Gool².* 1) epidemiology and biostatistics, Erasmus University, Rotterdam, the Netherlands; 2) department of neurology, Academic Medical Centre, Amsterdam, the Netherlands.

In the pathogenesis of Creutzfeldt-Jakob Disease (CJD) the prion protein plays a key role. The prion protein gene contains a polymorphism region at codon 129, coding for methionine (M, allele frequency in the population 64.4%) or valine (V, 35.6%). This study aims to evaluate the diagnostic value of the common codon 129 polymorphism in diagnosing sporadic and variant CJD. Literature reports on codon 129 genotypes were identified by a Medline search. Studies with data on probable or definite CJD patients and healthy controls, both of caucasian origin, with at least ten subjects were included. A Bayesian analysis was performed to explore the value of routinely genotyping suspected CJD patients. We found 11 articles fulfilling our inclusion criteria, resulting in a group of 670 controls, 935 sporadic and 49 variant CJD patients. Calculated likelihood ratios for sporadic CJD were 1.76 for MM, 0.27 for MV and 1.29 for VV and for variant CJD 2.38 for MM and .00 for MV and VV. Application of Bayes theorem showed that in sporadic CJD MV genotype and in variant CJD MV and VV strongly argue against a diagnosis of CJD. Our study shows that a common polymorphism may be useful in reducing the probability of CJD.

Evaluation of polymorphisms related with thrombogenesis in Behcet's Disease. *P. Bayrak Toydemir¹, A.H. Elhan², A. Tukun¹, M.R. Toydemir¹, A. Gurler³, A. Tuzuner⁴, I. Bokesoy¹*. 1) Dept Medical Biology, Division of Medical Genetics, University of Ankara, Faculty of Medicine, Ankara, TURKEY; 2) Dept Biostatistics, University of Ankara, Faculty of Medicine, Ankara, TURKEY; 3) Dept Dermatology (Behcet Center), University of Ankara, Faculty of Medicine, Ankara, TURKEY; 4) Dept General Surgery, University of Ankara, Faculty of Medicine, Ankara, TURKEY.

First described by Hulusi Behcet in 1937, Behcet's Disease (BD) is a multisystemic disease with unknown etiology. It is characterized by aphthous ulcerations and uveitis, and thrombotic tendency is commonly observed, deep venous thrombosis (DVT) being the most common form. Hyperactivity in the coagulation pathway, hypoactive anticoagulation mechanisms and fibrinolysis defects may cause thrombogenesis and mutations of the genes involved in these pathways may cause predisposition for thrombogenesis. Among these genes we evaluated possible roles of methylenetetrahydrofolate reductase (MTHFR) gene C677T, factor V (FV) gene G1691A, and prothrombin gene G20210A mutations in BD patients. 30 BD patients with DVT, 30 BD patients without DVT, 30 patients with only idiopathic DVT and 100 healthy individuals were studied with restriction fragment length polymorphism (RFLP) analysis. The frequencies of these mutations, separately and in combinations, for each group are evaluated. We did not find any significant difference in frequency of prothrombin gene G20210A mutation between control and patient groups. MTHFR gene C677T mutation did not increase DVT risk neither alone nor when combined with FV G1691A mutation. FV G1691A mutation was found to be a risk factor for DVT. Also an association between FV G1691A mutation and BD is likely however this mutation did not increase the DVT risk in BD patients. Although thrombotic tendency is one of the major characteristics of BD, we did not find any association between these three thrombogenic mutations and thrombogenesis in BD. It is possible that immune mediated vasculitis may be the primary event which eventually leads to thrombotic complications of BD.

The Association between Variations of Candidate Genes Related to Blood Pressure Regulation and Essential Hypertension in Korean Population. *B.Y. Kang¹, k.T. Kim¹, Y.H. Han¹, M.Y. Oh², Y. Namkoong³, S.S. Hong⁴, J.H. Shin⁴, C.C. Lee⁴.* 1) Seoulin Bioscience Institute, Seoulin Bioscience Co. Ltd, Seoul, Korea; 2) Department of Biology, Cheju National University, Cheju, Korea; 3) Department of Biology, Kangreung National University, Kangreung, Korea; 4) School of Biological Science, Seoul National University, Seoul, Korea.

Essential hypertension is considered to be caused by a complicated combination of genetic and environmental factors. The aim of present study was to examine the relationship between essential hypertension and variations of genes that encode proteins having an influence on the blood pressure regulation. Genotypes of 6 candidate genes (angiotensinogen (AGT), angiotensin I converting enzyme (ACE), angiotensin II type1 receptor (AT1R), bradykinin B2 receptor, kininogen and atrial natriuretic peptide (ANP) genes) were analyzed by polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) method. ScaI RFLP of the ANP gene was significantly associated with essential hypertension. Also, individuals with S1S1 homozygote of ScaI RFLP at the ANP gene had significantly higher systolic and diastolic blood pressure levels than other genotypes for these RFLPs. Allele frequencies of AGT, ACE and AT1R genes in Korean population were significantly different from those reported in Caucasian population. In conclusion, allele distributions of these genes observed in various population, may be useful to investigate the ethnic differences in blood pressure regulation and cardiovascular disease. Also, ScaI RFLP of the ANP gene may be useful as a genetic marker for essential hypertension in Korean population.

BLOOD PRESSURE, ANGIOTENSINOGEN AND ANGIOTENSIN I-CONVERTING ENZYME GENES IN YAKUT POPULATION. *O.V. Miloserdova¹, L.A. Tarskaia^{±3}, V.A. Spitsyn², S.A. Limborska¹*. 1) Human Molecular genetics, Inst Molecular Genetics, Moscow, Russia; 2) Medical Genetic Scientific Center, Moscow, Russia; 3) Institute of Health, Department of Biology, Republic Sauha (Yakutia).

Blood pressure (BP) may be influenced by several polymorphisms of the renin-angiotensin system, such as the angiotensinogen gene (ANG) and the angiotensin I-converting enzyme gene (ACE). We investigated the associations of these polymorphisms with blood pressure in Yakut population. This population is characterized as population with low frequency of cardiovascular disorders. The study group, which was entirely Yakut, consisted of 107 unrelated subjects (85 female and 22 male, mean age is 44.214.3 y.o.). We observed predominance I-allele and II- genotype in ACE (that is typical feature for Turkic populations) and increase of frequencies T-allele in comparison with Slavonic populations of Russia. We did not find any significant association between either I/D ACE or M235T AGT genotypes and the blood pressure. Results are presented in table. There was not any difference between male and female as well. On the basis of this data we can conclude none of the genotypes defined by the polymorphisms were associated with mean level of systolic BP (SBP), pulse BP (PBP) or diastolic BP (DBP) pressure.

	ACE			ANG		
	II	ID	DD	MM	MT	TT
SBP	126.48	130.46	119.29	127.03	133.91	120.69
PBP	46.46	49.52	40.57	47.53	50.22	44.27
DBP	79.86	81.35	78.36	79.51	83.67	76.42

Analysis of single nucleotide polymorphism in Japanese. *T. Morisaki^{1,2}, K. Toyama^{1,2}, M. Iwao¹, H. Matsunaga^{1,2}, H. Morisaki¹.* 1) Dept Bioscience, Natl Cardiovasc Ctr Res Inst, Suita, Osaka, Japan; 2) Dept Mol Pathophysiol, Osaka Univ Grad Sch Pharm Sci, Suita, Osaka, Japan.

Single nucleotide polymorphism (SNP) is the most frequent genetic variation, which is expected to facilitate large-scale association studies to identify genes susceptible to various common diseases. However, SNPs in certain population would not be useful to perform genetics studies in other population, since SNPs found in one population might not be found or be found at quite a different frequency in another population. Therefore, population-specific SNPs could give us important information for the study. Adenine nucleotide/nucleoside metabolism has an important role for maintaining cellular energy source, ATP, and adenosine. Recent study on the AMPD1 gene of this pathway revealed that the common variant in AMPD1, which was shown to be very common in Caucasians but not in Japanese, were associated with improved clinical outcome in patients with heart failure. Our study is aimed to collect SNPs in the loci of this pathway in Japanese, since they will be quite useful for studying the genes related with common diseases including heart failure. SNPs survey has been performed by using the methods of multiple-fluorescent primer based (MF) PCR SSCP (MF-PCR SSCP) and dHPLC. Hundred alleles from 50 Japanese volunteers were studied for each amplicon. First, as a model for SNPs survey, we have screened three AMPD loci (about 16 kb in total) and found 25 novel SNPs, including one non-synonymous cSNP, but not found C34T allele in AMPD1 that is quite common in Caucasians. Next, we have screened 16 other genes by the methods of dHPLC and MF-PCR-SSCP, and have found 54 SNPs including 6 non-synonymous cSNPs. All of these candidate SNPs have been confirmed by direct sequencing. These SNPs found in Japanese are being used for the study to identify genes susceptible to various diseases.

No association between an MTHFR polymorphism and occurrence of aneuploidy. *B.D. Kuchinka¹, L.N.*

Oppenheim¹, L-J. Henderson¹, M.D. Stephenson^{1,2}, W.P. Robinson¹. 1) Dept Medical Genetics, Univ British Columbia, Vancouver, BC, Canada; 2) Dept Obstetrics and Gynaecology, Univ British Columbia, B.C.'s Women's Hospital and Health Centre, Vancouver, BC, Canada.

The C677T allele of a methylenetetrahydrofolate reductase (MTHFR) gene polymorphism has been reported as a possible risk factor for Down syndrome (James, 1999). It was hypothesized that maternal meiotic nondisjunction might be associated with abnormal folate metabolism due to DNA hypomethylation. Furthermore, this MTHFR variant has been reported to be associated with hyperhomocysteinemia and increased thrombotic tendency which could, in turn, play a role in recurrent spontaneous abortion (RSA). Contradictory results have been presented regarding mutation in the MTHFR gene among women with RSA. To further investigate the possible association between MTHFR and aneuploidy, two groups of women were evaluated for MTHFR genotype: 1) 72 mothers of children with Prader-Willi Syndrome (PWS) due to uniparental disomy arising from a meiotic segregation error 2) women experiencing RSA, defined as 3 or more consecutive pregnancy losses. Frequency of the MTHFR C677T variant was evaluated in cases with aneuploid and euploid losses separately. The frequency of homozygotes and heterozygotes for the C677T allele in mothers of children with UPD15 was C/C 0.44, C/T 0.44, T/T 0.11 (N=72). This was not significantly different from the frequency observed in the fathers (used as controls) of C/C 0.41, C/T 0.46, T/T 0.13 (N=56). These observed frequencies were also similar to what we observed in the RSA group as a whole: C/C 0.44, C/T 0.51, T/T 0.04 (N=223). Of these women, 32 had at least one karyotyped loss which was aneuploid and 25 had one or more karyotyped loss, none of which were aneuploid. A contingency test failed to detect a difference suggesting the type of loss is independent of genotype. No significant association between the MTHFR C677T gene mutation in mothers of aneuploid pregnancies was detected. This does not exclude that blood homocysteine levels might not independently contribute to aneuploidy risks.

Does dietary fat intake modify the effect of the peroxisome proliferator-activated receptor-g2 (PPARg2)

Pro12Ala variant on obesity? *W.-C. Hsueh¹, S.A. Cole¹, B.A. Beamer², A.R. Shuldiner³, B.D. Mitchell¹*. 1) Dept Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Dept Medicine, Johns Hopkins University, Baltimore, MD; 3) Dept Medicine, University of Maryland, Baltimore, MD.

Previous studies have reported significant associations between obesity and the Pro12Ala variant of PPARg2. Fatty acids are ligands for PPARg and studies in rodents suggest that dietary fat intake regulates the expression and activity of PPARg. We hypothesized that the Pro12Ala variant would be more strongly associated with obesity in humans who consume a high-fat diet compared to those who consume a low-fat diet.

Analyses were conducted in 778 subjects from 23 large Mexican American families (mean age 38.1±16.1 years). Dietary fat intake was assessed using a food frequency questionnaire. The effect of fat intake was assessed by comparing the effect of the variant on obesity between subjects in the upper and lower tertiles of the dietary fat distribution. Statistical analyses were conditioned on the pedigree structures in order to account for the correlations among family members and statistical significance was evaluated by the likelihood ratio test adjusting for age and sex.

The Pro12Ala variant was associated with obesity (BMI, waist circumference and leptin levels) in this population. This association was stronger among those with higher saturated fat intake ($p = 0.03$ for PPAR Ala allele*diet interaction on leptin levels), and among those who derived a higher proportion of calories in their diet from fat ($p = 0.047$ for PPAR Ala allele*diet interaction on waist). These results provide modest support for the hypothesis that the effects of PPARg may be modulated in part by dietary fat intake. (Supported by HL 45522).

Haplotypic analysis of CD81 gene, the putative receptor for hepatitis C. *T.M. Marquardt, M. Odell, K. Kolodziejczak, C. Bertucci, C. Ton.* Genome Center, University of Washington, Seattle, WA.

Intro/Background Hepatitis C virus (HCV) is a widespread health problem affecting nearly 3% of the population worldwide. It has recently been shown that HCV binds to the major extracellular loop of CD81, suggesting a mechanism for viral entry into the cell. **Aims** A genetic analysis of the CD81 gene to ascertain its ethnic haplotype distribution. This was achieved through a deep search for polymorphisms in CD81 via the screening of about 300 individuals from 20 ethnic groups. **Significance** Since there is evidence that CD81 is a cellular receptor for HCV, it is possible that genetic variation in CD81 gene structure, expression, and regulation could impact host resistance to the virus, or progression of the disease. This would be analogous to the protective effects of polymorphisms in CCR5, CCR2, and SDF1 against HIV-1. As the first haplotype analysis of CD81, our work may facilitate a better understanding of the varying response to HCV infection and treatment shown by different ethnic groups. **Method** Genomic sequencing of exon-derived PCR products. The study population included a diversity panel of 10 members each from 20 ethnic groups and a 100 member panel of African Americans (both from Coriell), and 30 Caucasians. Sequences were aligned using the programs Phred/Phrap, and polymorphic sites (SNPs) identified as deviations from the consensus sequence. We sequenced approximately 4 kbp of the CD81 gene, with a strong emphasis on the exons, flanking intronic DNA, and splice sites. **Results and Discussion** We found a total of 27 polymorphic sites, including 3 coding SNPs (one non-synonymous) and 3 intronic SNPs within 10 bp of consensus splice sites. The non-synonymous coding SNP is located in exon 3 near a conserved transmembrane site, and results in conversion of an alanine to threonine; this creates the possibility for a functional change in the protein. Taken together, these SNPs define approximately 3 haplotypes, generally distributed along ethnic lines; 1 was present among the African-American populations but was not observed in the Caucasians. Other ethnicities also possessed SNPs that were not shared with other groups.

cSNP analysis of the *ABCA1* gene: the R219K variant is associated with a blunted age-modulated increase in HDL cholesterol and decreased coronary artery disease (CAD). *H.O.F. Molhuizen*¹, *S.M. Clee*², *A.H. Zwinderman*³, *J. Engert*⁴, *K.Y. Zwartz*², *K. Roomp*⁵, *A. Brooks-Wilson*⁵, *T. Hudson*⁴, *J.W. Jukema*³, *J.J.P. Kastelein*¹, *M.R. Hayden*². 1) Academic Medical Centre, Amsterdam, The Netherlands; 2) Centre for Molecular Medicine & Therapeutics, Vancouver Canada; 3) Leiden University Medical Centre, Leiden, The Netherlands; 4) McGill University, Montreal Canada; 5) Xenon Genetics Inc., Vancouver Canada.

We have recently shown that mutations in the *ABCA1* (*ABCI*) gene are associated with decr. plasma HDL cholesterol and incr. risk of CAD. However the contribution of frequent variation in the *ABCA1* gene to HDL levels and CAD risk in the general population is unknown. We have identified 15 cSNPs within the *ABCA1* gene, and have examined the effects of 9 non-conservative changes in a cohort of 804 Dutch males (REGRESS). Carriers of the R219K (G1051A, 47% in Caucasians) had significantly decr. CAD (mean segment diameter: 2.77 ± 0.37 vs 2.70 ± 0.37 , $p=0.01$ & minimal obstructive diameter: 1.80 ± 0.36 vs 1.73 ± 0.35 , $p=0.003$), and decr. events (12 vs 17%; OR=0.66, $p=0.04$) compared to non-carriers (NC). Initial assessment did not find any difference in lipid levels between carriers and NC. However, age is an important modifier of the phenotypic expression of *ABCA1* genetic variation. Striking age-related differences were seen. HDL was significantly incr. in carriers <50yrs than in NC <50yrs (0.93 ± 0.21 vs 0.87 ± 0.17 , $p<0.05$). In NC, HDL incr. significantly with age ($p<0.001$; mean HDL > vs < median age (57yrs): 0.96 ± 0.24 vs 0.88 ± 0.19 mmol/L, $p<0.001$), as in the general population, whereas there was no correlation between HDL and age in carriers ($p=0.04$ vs NC; mean HDL > vs < median age: 0.94 ± 0.23 vs 0.92 ± 0.24 , $p=0.37$). Thus for most of their lives, carriers have incr. HDL compared to NC, associated with a decr. risk of CAD. The R219K was found at higher carrier frequencies within Black and Chinese populations (98&67% respectively), suggesting that *ABCA1* genetic variation may account for part of the incr. HDL levels seen in these populations compared to Caucasians. These data suggest that variation within the *ABCA1* gene may be an important determinant of plasma HDL and CAD risk in the general population.

Polymorphisms linked to Factor IX gene in Korean population. *S.H. Shim¹, Y.H. Cho¹, H.K. Seo¹, J.H. Kim¹, C.H. Lee¹, J.S. Shin², S.R. Chung², H.J. Lee³*. 1) Department of Medical Genetics, College of Medicine, Hanyang University, Seoul 133-791, Korea; 2) Department of OB&GY, College of Medicine, Hanyang University, Seoul 133-791, Korea; 3) Department of Obstetrics and Gynecology, School of Medicine, Eulji University, Seoul 139-711, Korea.

Linkage analysis is a very useful method for prenatal diagnosis of Hemophilia B, especially when a mutation was not identified. Seven polymorphic markers were evaluated in Korean populations. Subjects of this study was 100 healthy Korean women (200 X-chromosomes). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to detect SalI, MseI, NruI, DdeI, XmnI, TaqI and HhaI polymorphisms. In SalI system 18 subjects were SalI(-/-), 35 Sal(+/-) and 47 SalI(+/+). SalI (-) allele showed the frequency of 0.355 and SalI(+) allele 0.645. MseI(-) allele was 0.645 in frequency and MseI(+) allele was 0.355. SalI and MseI polymorphisms were in complete linkage disequilibrium. And no increase was expected in overall heterozygosity with these two polymorphisms. In NruI system, 72 subjects were NruI(-/-), 27 NruI(+/-) and 1 NruI(+/+). NruI(-) allele frequency was 0.855 and NruI(+) was 0.145. There was no polymorphism of DdeI, XmnI and TaqI marker systems in Korean population. In HhaI polymorphism, 63 subjects were HhaI(-/-), 34 HhaI(+/-) and 3 HhaI(+/+). Allele frequencies were estimated that HhaI(-) is 0.82 and HhaI(+) is 0.18. Thus only SalI, NruI and HhaI polymorphisms are useful for the diagnosis of hemophilia B in Korean population. Expected heterozygosity for above 3 polymorphic markers was estimated to be 0.723, and 71 female subjects were heterozygous for at least one marker system. Korean population showed relatively low extent of polymorphisms compared to Caucasians, Blacks and Japanese.

Estimation of allele frequencies of SNPs by a quantitative SSCP analysis of pooled DNA. *T. Tahira, A. Suzuki, K. Higasa, T. Sasaki, S. Baba, Y. Kukita, K. Hayashi.* Genome Analysis, Inst Gen Info, Kyushu Univ, Fukuoka, Japan.

In association studies using SNPs as genetic markers, allele frequencies of the SNPs in groups of individuals should be accurately estimated. Allele frequencies are reliably estimated only when it is examined among a large number of individuals, and a realistic approach for that is to quantitatively analyze pooled DNA samples. We have been proposing PLACE-SSCP (PCR products are fluorescently post-labeled and separated by an automated capillary electrophoresis system in SSCP conditions), as a method for analysis of pooled DNA. In this method, alleles are separated into peaks, and their frequencies can be quantified by their relative peak heights. We have established a strategy to compensate the biased amplification/labeling between alleles by using heterozygotes and also to evaluate measurement errors. According to this strategy, measurement errors in s.d. is less than 0.018, which is equivalent to a sampling error of 1,500 individuals. Then, we examined effectiveness of this method in estimation of allele frequencies of candidate SNPs in two groups of individuals of different ethnic origin (Japanese and Caucasian (CEPH parents)). We analyzed 235 candidate SNPs in chromosome 6 and 11 in a public database (Human SNP database, Wang et al., 1998). Among the 185 SNPs that were verified by direct sequencing of individual DNAs, 162 (88%) were detectable by PLACE-SSCP and 123 (76%) of them were quantified with confidence. SNPs informative (SNPs with allele frequencies greater than 10%) in both groups constitute 58% of SNPs quantified. Other 25% of quantified SNPs were informative in only one group. Since SNPs not confirmed in our strategy are either monomorphic or with low allele frequencies, fraction of informative SNPs among candidate SNPs in a certain population might be less than 50%. This study shows that PLACE-SSCP should be very effective tool in selecting informative SNPs from existing tens of thousands of candidate SNPs. For this purpose, we have been developing high-throughput PLACE-SSCP analysis in which electrophoresis is performed in capillary arrays.

Genetic Diversity of Apolipoproteins in North Indian Populations. *P. Singh¹, M. Singh¹, S.S. Mastana²*. 1) Department of Human Biology, Punjabi University, Patiala, Punjab, India; 2) Human Genetics Lab., Department of Human Sciences Loughborough University Loughborough, LE11 3TU, UK.

Various epidemiological studies have showed that genes coding for apolipoproteins (APOB, APOE, Apo AI-CIII-AIV and CI-CII-E gene clusters, Lp (a)) are candidates for determining genetic variation in plasma lipid levels and risk of atherosclerosis. Most of these studies evaluating the risk of atherosclerosis vis-a-vis apolipoproteins were carried out mainly in European populations. Such types of polymorphic studies are limited in Indian populations. We have analysed APOE, APOC-II, APOH, and APO-AIV in 9 endogamous populations from the North Indian State of Punjab. 1075 individuals belonging to Brahmin, Bania, Jat Sikh, Khatri, Rajput, Scheduled Castes, Lobanas, Ramdasia and Ramgarhia castes were genotyped using isoelectric focusing and two-dimensional gel electrophoresis. The overall level of polymorphism in these populations is extensive and comparable to many Caucasian populations. A number of interesting genetic features and clines emerged from our extensive analyses. The world's lowest APOE*4 allele frequency was observed in Ramgarhia, while Ramdasia had the highest APOA-IV*2 allele frequency (0.093). The frequency of APOCII-2 was also observed to be the highest in Punjabi populations. Multivariate analysis (Correspondence analysis, multidimensional scaling analysis) of the Punjabi and the world populations highlights the potential of these markers for human genetic diversity studies in addition to medico-genetic implications.

cSNPs in the genes for the neuronal nicotinic acetylcholine receptor subunits CHRNA4 and CHRNB2. *C.C. Ton, K. Kolodziejczak, D. Zlatanova, C.F. Bertucci.* Dept Medical Genetics, Univ Washington, Seattle, WA.

The 11-member family of neuronal nicotinic acetylcholine receptors (nAChR) has been implicated in a wide range of neurological, psychiatric, and behavioral processes. Examples include the role of CHRNA4 in autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), CHRNA7 in the P-50 auditory evoked potential deficit, a potential endophenotypic marker for schizophrenia, and of CHRNB2 in nicotine addiction. Thus knowledge of the identity and population distribution of cSNPs within the nAChRs can be of great value in the genetic dissection of such disease states. We have conducted an exhaustive search for possible functional polymorphisms in genes CHRNA4 and CHRNB2 by means of gel-based, direct genomic resequencing of all exons, segments of flanking introns and UTRs. The DNA templates for this deep search were derived from the extended Human Diversity panel available from Coriell: 10 individuals each from 17 ethnic groups, 30 Northern Europeans, and 100 African Americans (for total $n=300$). The analyses on CHRNA4 and CHRNB2 have so far revealed a total of 9 non-synonymous substitutions (minor allele frequencies $q < 0.03$), 12 synonymous substitutions ($q < 0.15$), and 6 intronic SNPs located within 10 bp of a splice consensus. The non-synonymous substitutions all occurred within the extracellular domains of the receptors; none were found near the transmembrane domains where the CHRNA4 ADNFLE mutation was originally reported. The probable haplotypic organization of these SNPs have also been deduced, revealing distinct patterns of population stratification. Strikingly, all of the non-synonymous substitutions discovered appeared to be private ones i.e. not shared across ethnic groups. This raises the possibility that pharmacogenetic variations in agonist/receptor (e.g. nicotine/nAChR) interactions could lead to marked ethnic difference in drug response. This information is being applied to studies on smoking predisposition and cessation, schizophrenia, and other neurological processes.

Population history affects the expected number of ancestral chromosome segments. *N.H. Chapman¹, J. Crumley⁴, T.M. Fujiwara^{3,4}, K. Morgan^{3,4}, E.A. Thompson^{1,2}.* 1) Departments of Biostatistics and; 2) Statistics, University of Washington, Seattle, WA; 3) Departments of Human Genetics and Medicine, McGill University; 4) Montreal General Hospital Research Institute, Montreal, QC.

An isolated population is a group of individuals who are descended from a founding population who lived some known time ago. If the N founding individuals are assumed to be non-inbred and unrelated, their genetic material can be represented as $2N$ distinct ancestral chromosomes. A chromosome sampled from the modern population will be composed of segments of some of those $2N$ ancestral chromosomes. A modern population in which chromosomes are made up of a few long segments will exhibit linkage disequilibrium due to founder effect over longer distances than a population in which the chromosomes are made up of many short segments. It is therefore useful to know the number of segments expected in a chromosome sampled from a modern population.

The histories of isolated human populations differ in terms of age, growth patterns, and internal structure. Assuming random mating, we study analytically the effects of these factors on the expected number of segments in a chromosome randomly selected from a modern population. We demonstrate that the type of growth a population has experienced can dramatically influence the expected number of segments. Over long time periods, exponential growth can result in many fewer segments than slow linear growth. Population subdivision also affects the expected number of segments. Assuming that subdivision occurs randomly with respect to kinship, we show that a larger number of internal subdivisions results in a smaller number of expected segments. Subdivision that occurs earlier in the history of the population results in a greater reduction in the expected number of segments. We also develop an approximation to the variance of the number of segments, in order to assess the importance of the above results. Finally, we study by simulation the effects of non-random mating and non-random subdivision in an isolated population, using the Hutterite Brethren as an example.

Association of an intronic variant of peroxisome proliferator-activated receptor- γ coactivator-1 (PGC-1) with decreased body mass index (BMI) in Caucasians and interaction with the Trp64Arg variant of the β -3 adrenergic receptor. Z. Fan¹, T.I. Pollin¹, D. Gong¹, M.J. Garant¹, J.C. McLenithan¹, B.M. Spiegelman², E.T. Poehlman³, A.R. Shuldiner¹. 1) University of Maryland, Baltimore, MD; 2) Harvard University, Boston, MA; 3) University of Vermont, Burlington, VT.

PGC-1 has been demonstrated to be a key factor in the stimulation of adaptive thermogenesis, a component of the metabolic defense against obesity. Using single-stranded conformational polymorphism analysis, we identified a common variant in intron 2 of the PGC-1 gene (C \rightarrow A, 52 nucleotides from the splice acceptor site). In a Caucasian cohort of 897 women and 67 men, the allele frequency was 0.41. PGC-1 variant homozygosity was significantly associated with decreased age- and sex- adjusted BMI (wild-type homozygotes and heterozygotes: BMI=33.2 \pm 0.3 kg/m², n=820; homozygous variant: BMI=31.2 \pm 0.7 kg/m², n=145, p=0.005).

Stimulation of the β -3 adrenergic receptor (β 3AR) has been shown to induce PGC-1 expression. To test for an interaction between the PGC-1 variant and the previously identified Trp64Arg variant in β 3AR, the cohort was divided into four groups with age- and sex- adjusted BMI as follows: (1) those with neither variant genotype (n=723): BMI=33.2 \pm 0.3 kg/m², (2) β -3 variant only (heterozygotes and homozygotes) (n=96): BMI=33.3 \pm 0.8 kg/m², (3) PGC-1 variant homozygotes only (n=121): BMI=30.7 \pm 0.7 kg/m², and (4) both variant genotypes (n = 24): BMI=33.4 \pm 1.6 kg/m² (p=0.02 for overall null). In pairwise comparisons, the PGC-1 variant was significantly associated with decreased BMI in the absence of the β -3 variant (p=0.009) but not in the presence of the β -3 variant (p = 1.000). The PGC-1 variant significantly decreased the odds of obesity (defined as BMI \geq 30 kg/m²) in the absence of the β -3 variant [OR=0.60, 95% CI: (0.41, 0.89), p=0.01] but not in the presence of the β -3 variant [OR=2.05, 95% CI: (0.75, 5.63), p=0.15] (p=0.02 for homogeneity of the odds ratios).

These data suggest that the PGC-1 variant acts protectively against fat accumulation, possibly through increased expression of PGC-1 which requires functioning β -3-adrenergic receptors.

Nucleotide and sequence haplotype diversity at the human apolipoprotein AII (*APOA2*) locus: significant deficit of polymorphism in an African-American sample. *S.M. Fullerton*¹, *K.M. Weiss*¹, *A.G. Clark*¹, *S.L. Taylor*², *J.*

*Stengård*³, *E. Boerwinkle*⁴, *C.F. Sing*⁵, *D.A. Nickerson*². 1) Depts of Anthropology & Biology, Pennsylvania State Univ, University Park, PA; 2) Dept of Molecular Biotechnology, Univ of Washington, Seattle, WA; 3) National Public Health Institute, Dept of Epidemiology & Health Promotion, Helsinki, Finland; 4) Human Genetics Center, Univ of Texas Health Science Center, Houston, TX; 5) Dept of Human Genetics, Univ of Michigan Medical School, Ann Arbor, MI.

In an effort to identify genetic variation that may contribute to inter-individual differences in heart disease risk, we have conducted a survey of DNA sequence diversity at the human apolipoprotein AII (*APOA2*) locus. A 3.3 kb region, encompassing the whole of the *APOA2* gene and associated 5' and 3' flanking regions, was surveyed for 24 individuals from each of 3 populations: African-Americans from Jackson, MS, non-Hispanic whites from Rochester, MN, and Finns from North Karelia, Finland. Fifteen variable sites were identified in the combined sample (14 single nucleotide polymorphisms and 1 multi-allelic microsatellite variant) and the SNP sites were inferred to segregate as 10 distinct sequence haplotypes. Unexpectedly, 2 of the 10 haplotypes were found at high frequency in all 3 samples, constituting over 75% of the 144 chromosomes surveyed. As the 2 closely-related haplotypes make up most of the African-American sample, diversity in that sample was particularly low, with fewer than the expected number of variable sites observed relative to variation found in the other samples ($P < 0.02$), or as predicted by patterns of polymorphism and divergence at other human loci (e.g. vs. Jackson *LPL* sample, $P < 0.02$). This marked reduction in diversity is consistent with the rapid rise in frequency of the common haplotypes, possibly under the influence of positive natural selection. As no nonsynonymous variant was found in the *APOA2* gene, the site(s) which may have served as the target of selection are either regulatory in nature or fall outside the genomic region surveyed. (This work was supported by NHLBI grants HL39107, HL58238, HL58239, and HL58240).

STUDY OF COMPLEX TRAITS USING ISOLATED INBRED HUMAN POPULATIONS: THE

CARLANTINO PROJECT. *P. Gasparini¹, P. Palancia², F. Lo Vecchio¹, M. Villella¹, A. Villella², M. Urbano², G. Grosso², F. Abiuso², C. Di Giovine¹, A. D'Adamo¹, P. Stanziale¹, F. Bertoldo³, L. Zelante¹.* 1) Medical Genetics Service, IRCCS-CSS, Rotondo, Italy; 2) ASL-Foggia3; 3) Verona University.

The use of isolated populations to reduce disease heterogeneity of complex disorders has already proved to be useful. The key to the success of this study is the use of a geographically and culturally isolated population, which makes it possible to identify markers in linkage disequilibrium with the disease. The relatively recent founding of the population makes it possible to detect linkage disequilibrium over a larger genetic interval as compared to an older diverse population. For complex traits the use of inbred populations can increase our ability to detect association by comparison of genotype distribution between cases and controls. It is expected that an association can be detected with a smaller sample of patients in an inbred population than in a panmictic one. We identified a small village, named Carlantino, characterized by 1400 inhabitants and located in Southern Italy. The village has been settled 5 centuries ago by few founders and during last century the endogamy has been calculated to be 99.5%. Three different surnames accounts for the majority of the living people. Birth registers are available from 17th century, and in a more detailed way from 19th century. The project is divided as follows: step 1: a full clinical examination of all inhabitants including anamnesis, blood pressure measurements, cardiac evaluation by electrocardiography and bone mineral density evaluation by ultrasonography, a complete clinical chemistry evaluation, step 2: development of DNAs and sera banks, step 3: inclusion of all data in a database. Step 1 and 2 have been already completed, while the inclusion of all the data (historical, clinical, biochemical and instrumental) in the database is now in progress (Step 3). Construction of DNA haplotypes of Y and X chromosomes, and of mitochondrial DNA suggested the presence of 6 couples as founders. Preliminary epidemiological data indicate an increased frequency of osteoporosis, hypercholesterolemia, heart stroke pedigrees, cancer families, and miopia.

Mitochondrial lineages in the Roma. *D. Gresham*¹, *G. Passarino*², *I. Tournev*³, *R. de Pablo*⁴, *V. Kucinskas*⁵, *F. Calafell*⁶, *L. Kalaydjieva*¹. 1) Centre for Human Genetics, Edith Cowan University, Joondalup, W.A., Australia; 2) Genetics Department, Stanford University Medical School, Stanford, CA; 3) Medical University, Sofia, Bulgaria; 4) Unidad de Inmunología, Clínica Puerta de Hierro, Madrid, Spain; 5) Vilnius University Hospital, Human Genetics Centre, Lithuania; 6) Unitat de Biologia Evolutiva, Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain.

The Roma comprise some 8-10 million people resident throughout Europe. Based on linguistics, the Roma are believed to have originated in India. Here we report on maternal lineages in Roma populations using mtDNA. In a sample of 169 individuals, from five Romani populations in Bulgaria and populations from Spain and Lithuania, we performed RFLP analysis and sequencing of the hypervariable sequence 1 (HVS1).

Haplogroup M (+10394*Dde*I, +10397*Alu*I) was found to occur at a frequency of 22.5% in the total sample. Within each population, the frequency of haplogroup M ranged from 16% to 33.3%. Haplogroup M occurs at high frequency in Indian and East African populations. However, East African haplogroup M is characterized by the D-loop motif (16129, 16189, 16223, 16249, 16311) that is not found in our sample. A reduced median network was constructed in order to examine the phylogenetic relationship of mtDNA sequences in the Roma.

HVS1 sequences were compared to a database of published sequences. Unique sequences belonging to haplogroups M and X were found in the sample. Intermatch-mismatch distances were used to create a population tree that places the Romani populations as genetic outliers from other European populations and demonstrates significant distances between Romani populations.

This study demonstrates the common origins of socially and geographically separated Romani populations. The high frequency of Asian haplogroup M is consistent with the Indian origins of the Roma. At the same time, population fragmentation has resulted in pronounced genetic differentiation between Romani groups.

The molecular definition of haplogroup B: significant heterogeneity revealed by complete sequence analysis of Asian and Native American haplogroup B mtDNAs. *M.D. Brown¹, S. Hosseini¹, R.I. Sukernik², Y.B. Starikovskaya², O. Derbeneva², A. Torroni³, D.C. Wallace¹.* 1) Ctr Molec Med/420B Dental Bldg, Emory Univ Sch Medicine, Atlanta, GA; 2) Institute of Cytology and Genetics, Russian Academy of Sciences, Novosibirsk, Russia; 3) Dipartimento di Genetica e Biologia Molecolare, Universita di Roma "La Sapienza," Rome, Italy.

Nearly all Native American mitochondrial DNAs (mtDNAs) are subsumed by five mtDNA lineages or haplogroups: A, B, C, D, and X. With the exception of haplogroup B, these mtDNA lineages are defined by a number of stable base substitution polymorphisms. Haplogroup B is characterized by a 9 base pair deletion in the COII - tRNA^{Lys} intergenic region, restriction site losses at nucleotide pairs (nps) 10394 (DdeI) and 10397 (AluI), and a transition at np 16189 in the mtDNA control region (CR). However, some of these markers are hypervariable in human populations. Further, some authors have subdivided this lineage into haplogroup B1 and B2, depending either on the presence of a HaeIII site at np 16517 or on the occurrence of a transition at np 16217. Thus, the genetic definition of mtDNA haplogroup B is unclear. To more precisely define and identify haplogroup B mtDNAs, we completely sequenced 10 haplogroup B mtDNAs from Asia and the Americas, which encompassed a wide geographic distribution. Both putative B1 and B2 subtypes were included. We found significant heterogeneity in haplogroup B sequences, which indicated that: (a) a suite of at least six mutational markers must be discerned to minimally identify haplogroup B mtDNAs, (b) key haplogroup B mutations can be prone to reversion events, (c) certain mutations clustered in some, but not all, B mtDNAs, and (d) distinct sublineages are likely. Overall, these data permit a more refined molecular definition of mtDNA haplogroup B, which, in turn, will aid in the analysis of the origins and migrations of ancient peoples harboring haplogroup B.

Population replacement in human prehistory as assessed by ancient mtDNA. *M.G. Hayes, D.H. O'Rourke.* Dept Anthropology, Univ Utah, Salt Lake City, UT.

Sharp transitions in the archaeological record are often postulated to represent either population replacement or cultural diffusion events. We have studied the genetics of two such transitions in the North American Arctic which provide contrasting results. In the Eastern Canadian Arctic a transition occurs approximately 1000 years ago with the replacement of the Dorset culture by the Thule culture. These two cultural complexes are substantially different in terms of their material remains, but not in their craniometrics. The opposite pattern characterizes a transition that occurs at approximately the same time in the Aleutian Islands. Here, there is good evidence for cultural continuity over the last 4000 years, and instead the transition is delineated by a brachycranial population (Neo-Aleut) replacing a dolichocephalic population (Paleo-Aleut). To assess the genetic relationships of pre- and post-transition populations ancient DNA was analyzed from relevant archaeologically recovered individuals. Four restriction site or length polymorphisms, which define a minimum of four ubiquitous Native American mitochondrial haplogroups (A through D), were amplified and electrophoretically scored for the presence or absence of the marker. In the Eastern Canadian Arctic, >25 individuals have been analyzed and the haplogroup frequency distributions of the Dorset (33% A, 67% not A) and Thule (100% A) are statistically significantly different from one another ($p=0.025$, Fishers exact test). In the Aleutian Islands, >35 individuals have been analyzed and the haplogroup frequency distributions of the Paleo-Aleut (50% A, 50% D) and Neo-Aleut (31% A, 59% D) are not statistically significantly different from one another ($p=0.428$, Fishers exact test). These results preliminarily suggest population replacement in the Eastern Canadian Arctic and population continuity in the Aleutian Islands. The inclusion of additional samples and the examination of HVR I sequences are currently underway to further investigate this phenomenon.

Single Nucleotide Polymorphisms: Effects of mutation, recombination and genetic drift. *M. Kimmel¹, R.*

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The evolutionary dynamics of Single Nucleotide Polymorphisms (SNPs) is important for understanding their distribution in human genome and their applications in population genetics, evolutionary biology, and disease-gene association studies. Mathematical and computer modeling provides a convenient framework to investigate these issues. Previously, we considered several models, which involved drift and mutation. Our results suggested that the frequency of recurrent and reverse mutations in SNPs might be considerably higher than thought and transition versus transversion nucleotide substitutions are not equi-probable, particularly at SNP sites at which recurrent mutations are detected. Currently, we have studied a mathematical model involving recombination between a pair of loci in addition to drift and mutation. Mutations are assumed to follow a general time-continuous Markov chain model, which includes finite as well as infinite allelic states, with possible asymmetry of mutation probabilities. We obtained explicit solutions to the equations for transient as well as equilibrium conditions. They make possible to describe the decay of linkage disequilibrium between two loci as a function of time, jointly with the accompanying changes of genetic diversity at these loci. Predicted patterns of linkage and diversity at a pair of SNP loci, using several models of mutation, are compared to observations gathered from published data. (Research supported by US Public Health Service Research grant GM 58545 from the National Institutes of Health).

Heritability of height and assortative mating in the Framingham Study. *N. Mukhopadhyay¹, D.N. Finegold¹, M. Larson², L.A. Cupples², R.H. Myers², R. Ferrell¹, D.E. Weeks¹.* 1) Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Boston University, Boston, MA.

Height is the quintessential polygenic trait in humans. Its clinical importance is emphasized by the fact that 302 entries in OMIM contain references to height or stature. Adult height is influenced by both genetic and environmental factors, as indicated by measurements of heritability ranging from 0.60 to 0.85. Variations in determination of heritability may depend on accuracy of the data collected, size of the population studied, and approach to the data analyses. We analyzed 7,989 measured individuals in 2,392 extended pedigrees from the Framingham Heart Study. These individuals were ascertained in two cohorts, the first ascertained in 1948 via a random sample of households in Framingham, MA, and the second Offspring cohort in 1971. Individuals in the original cohort have undergone biennial examinations since inception of the study, while individuals in the Offspring cohort have been examined every 4 years (except between examinations 1 and 2 with an 8 year gap). At each exam, height was measured with subjects standing erect with their heads in the Frankfort plane. Height data were carefully cleaned by checking multiple measurements for excess intra-individual variation. After cleaning, we used each individual's first height measured in the age range 20 to 55 to determine heritability. Heritability was estimated both by parent-offspring regression and by variance-component methodology, allowing for gender and cohort effects. The heritability was estimated to be 0.81. We also found evidence of assortative mating effects, with 1,575 spouse pairs having a correlation of 0.35. Our analyses of this large carefully studied population confirm the strong genetic effect on adult height. We are currently analyzing genome-wide marker data on the Framingham pedigrees in order to map loci influencing normal adult height.

D.N. Finegold and D.E. Weeks contributed equally to this work. The data are from the National Heart, Lung and Blood Institute's Framingham Heart Study at Boston University.

Microsatellite loci in the HLA-Class 1 gene region show weak evidence of overdominant selection. *S.R. Indugula¹, G. Sun¹, S. Chunhua¹, D. Smelser¹, R. Kaushal¹, H. Xu², M. Kimmel³, Y. Zhong², R. Chakraborty², R. Deka¹.* 1) Dept of Environmental Health, University of Cincinnati, Cincinnati, OH; 2) Human Genetics Center, Univ. Texas School of Public Health, Houston, TX; 3) Statistics Dept., Rice University, Houston, TX.

DNA sequence polymorphism as well as allelic diversity at immunologically detected markers in and around the HLA-class 1 gene region demonstrated that the polymorphisms at this region of the human genome is maintained by overdominant selection. To investigate whether or not microsatellites can also be used for such biological discovery, we genotyped over 1200 individuals from 22 diverse populations at seven CA-repeat loci (D6S265, D6S273, TAP1, MOG, DQCAR, DQCAR1, and D6S291), and studied several features of microsatellite allele size distributions at this gene region. Nearly 21% of the locus-population combinations of genotype distributions showed significant departure from the Hardy-Weinberg equilibrium. Over 35% of these tests showed excess heterozygosity, a feature not explained by population substructure of the sampled populations. Observed homozygosity values, however, conditioned on the number of alleles reside within the published 95% confidence regions prescribed by the infinite allele mutation model. Allele frequency spectrum (i.e., number of alleles with different number of copies in the samples) showed some evidence of increase in the number of alleles with intermediate frequencies. Large values of the imbalance index based on allele size variance and expected heterozygosity, together with excess number of rare alleles, signify that recent population size expansion following a bottleneck dominates the features of allele frequency distributions at these microsatellite loci, probably erasing signatures of natural selection. In summary, these data at best suggests a weak effect of overdominant selection (e.g., excess heterozygosity), and signifies the need of further theoretical formulations for detecting natural selection effects at microsatellite loci. (Research supported by US Public Health Service Research grants GM 45861, GM 41399, and GM 58545 from the US National Institutes of Health).

Overall linkage disequilibrium in 33 populations for highly informative multisite haplotypes spanning the HOXB gene cluster. *K.K. Kidd, V. Busygina, M.M.C. DeMille, W.C. Speed, V. Ruggeri, J.R. Kidd, A.J. Pakstis.* Dept Genetics, Yale University, New Haven, CT.

The homeobox B gene cluster, HOXB@, on 17q21-q22 extends more than 100kb with many polymorphisms (SNPs, InDels, STRPs) identified throughout a 97kb region from HOXB1 to HOXB9. Recently, we have completed typings and analyses for 5 of these polymorphisms in 33 population samples (1,765 individuals) from around the world. These results include a new 4bp InDel in HOXB3 giving heterozygosities ranging from .06 to .49 (median=.21) along with 4 previously published polymorphisms (3 RSPs, 1 STRP). The order and physical distances in kb, based on extensive sequencing across the region for these sites, is: SacI HOXB6 --3.2--(CA)_n HOXB6--20.5--MspI HOXB4--12.6--TaqI HOXB3--18.6--InDel HOXB3. The 5-site haplotypes based on these typings have a median heterozygosity of .70 in these samples (range, .28-.94). Six SubSaharan African and one African American population samples display the highest levels of genetic diversity using a variety of measures. 27 of the 116 5-site haplotypes found (of 224 possible) occur at common frequencies (>5%) in one or more of the populations studied thus far. For these five sites extending over a 55kb span, overall linkage disequilibrium (LD) is strong and statistically significant in all 33 populations ($p < .001$ in 31 pops; $p < .05$ in 2 pops). A general trend exists, despite large variation in some regions, such that the average r coefficient (Zhao et al., 1999, Ann Hum Genet 63:167-179) quantifying overall LD, computed for major geographical areas, increases with distance from Africa: Africa ($r = 2.39$), Europe and SW Asia ($r = 3.88$), East Asia ($r = 4.71$), Pacific Islands ($r = 3.50$), N.America ($r = 4.67$), and S.America ($r = 5.70$). A single haplotype, uncommon in SubSaharan Africa (range, .00-.09; median=.045), has a range of .20 to .85 (median=.50) in all 26 non-African populations and is the most common haplotype in 23 of them, supporting the idea of a single founding of non-African populations and a lower effective population size (N_e) for non-African than for African populations. This work was supported in part by GM57672 and SBR9632509.

Relative fitness of women with the mitochondrial DNA mutation 3243A>G. *J.S. Moilanen, K. Majamaa.*

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Deleterious point mutations in mitochondrial DNA (mtDNA) have been found in many human populations and always at a low frequency suggesting that the mutations are under a strong negative selection. It is assumed that this selection is caused by reduced genetic fitness of mutation carriers, but the fitness of carriers of any mtDNA mutation has not been determined. One of these mutations is 3243A>G, the most common cause of the syndrome of mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS). We estimated the reproductive disadvantage caused by 3243A>G by calculating the net reproductive rates of women in 16 families with this mutation. The families were ascertained in the population of the province of Oulu, Finland (population 452,367 in 1996). The net reproductive rates of women with the mutation and women in the general population were estimated during the period of January 1, 1973 to December 31, 1996. The net reproductive rate of mutation carriers was estimated by using the person-years method and Kaplan-Meier survival analysis, whereas annual net reproduction rates of the general population were obtained from official population statistics. The net reproduction rate calculated for the women with 3243A>G was 1.02 (0.75-1.29, 95% confidence interval). The annual net reproduction rate of the general population varied between 0.87-1.06, the median being 1.01. The mean of the age-specific fertility distribution was 28.0 years for the mutation carriers being similar to that in the general population. Thus, women with 3243A>G reproduced at the same rate as women in the general population suggesting that women with 3243A>G are generally affected only mildly or late in life. Consequently, the host-level selection against women harboring the 3243A>G mutation appears weak. The observed low frequency of this mutation in population could still be explained by a mild, uniform selection or selection that depends on the degree of the mutant mtDNA heteroplasmy. On the other hand, our results are also compatible with the hypothesis that the host-level selection is nonexistent.

No independent origin of modern humans in East Asia: A tale of 12,000 Y chromosomes. *L. Jin^{1,2,5}, Y. Ke², X. Song², P. Underhill⁵, C. Xiao⁵, S. Marzuki⁵, R. Deka⁵, M. Shriver⁵, J. Lell⁵, D. Wallace⁵, R.S. Wells^{3,5}, M. Seielstad^{4,5}, D. Zhu⁵, D. Lu², W. Huang⁵, R. Chakraborty¹, B. Su^{1,5}.* 1) Human Genetics Ctr, Univ Texas, Houston, TX; 2) Fudan University, China; 3) Oxford University, UK; 4) Harvard University; 5) The Y-Chromosome Consortium.

The continuity of hominid fossils in East Asia, particularly in China and Indonesia, has been presented as strong evidence supporting the multiregional hypothesis where the independent origin(s) of modern humans are assumed. To search for evidence of a possible independent origin, 12,000 male individuals (over 11,000 were from East Asia, Southeast Asia, and Indonesia) have been sampled and typed at three Y biallelic markers (M1, M89, and M130). We show that each individual involved in this study carries a mutation at one of the three aforementioned sites. These three mutations (M1+, M89+, M130+) coalesce to another mutation (M168+), which was introduced into human populations about 60,000 years before the present. Furthermore, it can be shown that M168+ was originally derived from Africa, as no single individual was found that carries a lineage older than M168+ outside Africa. In other words, all 11,000 Y chromosomes sampled from East Asia and Indonesia, with no exception, were originally derived from the M168+ lineage from Africa. Therefore, we conclude that even a very minor contribution of in-situ hominid origin cannot be supported by Y chromosome evidence.

The Dual Origin and Siberian Affinities of Native American Y Chromosomes. *J.T. Lell¹, R.I. Sukernik², Y.B.*

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Over 500 Y chromosomes from 28 native populations representing South, Central, and North America as well as from southern and eastern Siberia were analyzed to investigate the origin and Old World affinities of Native American paternal lineages. Fourteen distinct haplogroups were defined by 11 biallelic markers, and 111 haplotypes were defined by the addition of data from four microsatellite loci. The major Native American founding Y chromosome haplogroup was defined by the biallelic markers M89/M9/M45/M3 (haplogroup M3). The great majority of non-M3 Native American Y chromosomes were defined by the markers M89/M9/M45 (haplogroup M45). Ancestors of haplogroup M3 were found in southern Middle Siberia, but the M45 haplotypes had dual affinities with southern and eastern Siberia. A third Native American founding lineage, defined by the RPS4Y np711 C->T transition (haplogroup S4Y-T) was also present in the eastern Siberian populations of Kamchatka and the Lower Amur River basin, as well as in the northern Amerind and Na-Dene Indian populations of North America. Based on these data, we propose a dual migration model for the founding of present-day Native American Y chromosomes. The first migration originated in the Upper Yenisey/Western Baikal region of southern Siberia and contributed haplogroups M3 and M45 to the Amerinds of North, Central, and South America. The second migration originated in the Lower Amur/Okhotsk coast region of eastern Siberia and contributed haplogroup S4Y-T as well as distinct M45 haplotypes to the Amerinds and Na-Dene Indians of North America.

Sequence variation and linkage disequilibrium at the fragile X syndrome locus. *D.J. Mathews¹, R. Hudson², E. Eichler¹, A. Chakravarti¹.* 1) Department of Genetics, Case Western Reserve University, Cleveland, Ohio; 2) Department of Ecology and Evolution, University of Chicago, Chicago, Illinois.

The human genome project will provide a reference sequence, which can serve as the template for resequencing studies that aim to discover and interpret the record of common ancestry that exists in extant genomes. To understand the nature and pattern of variation and linkage disequilibrium which comprise this history, we present a study of an ~68 kb region of the X chromosome, sequenced in a sample of 24 humans (worldwide sample) and great apes.

25 polymorphic sites and two insertion/deletions were found across an average of ~31 kb compared among humans, spanning the unique sequence at the fragile X locus (FMR1). There were 74 fixed differences between the humans and the great apes. Within humans, 11 unique haplotypes were identified; Africans are the only geographic group that does not share any haplotypes with other groups. Nucleotide diversity, q , for this sample is $2.42 \times 10^{-4} \pm 9.71 \times 10^{-5}$, with no significant variation among groups. The mutation rate, m , is 6.38×10^{-10} /bp/yr, giving an ancestral population size of ~6800 and a time to the most recent common ancestor of ~400 Kyr BP. Linkage disequilibrium (LD) at the FMR1 locus was evaluated both by conventional LD analysis and a new statistic, S (the length of segment shared between any two chromosomes, identical by descent). Both methods revealed significant LD in the region. Parsimony analysis revealed two main clades and suggests that the four major human geographic groups are distributed throughout the phylogenetic tree and within each major clade. An African sample appears to be most closely related to the common ancestor shared with the three other geographic groups.

These data show that the oldest human lineages do not necessarily have only one origin. Furthermore, in order to develop the most accurate view of our history, many long contiguous regions, such as the one presented here, must be studied.

ALU Genetic Diversity in Indian Populations. *S.S. Mastana¹, S.S. Papiha², P. Singh⁴, K. Das³, A. Pacynko¹, P. Fisher¹, N. Malik¹, M. Das³, P.H. Reddy⁵.* 1) Dept Human Sci, Human Gen Lab, Loughborough Univ, Loughborough, England; 2) Department of Human Genetics University of Newcastle upon Tyne Newcastle Upon Tyne; 3) Anthropometry and Human Genetics Department, Indian Statistical Institute, Calcutta, India; 4) Department of Human Biology, Punjabi University Patiala Punjab, India; 5) Neurological Sciences Institute Oregon Health Sciences University 1120 NW Avenue Portland, OR 97209-1595. USA.

ALU polymorphisms provide a useful tool to population geneticists for understanding the population dynamics that have occurred over time. We report here a study of Six Alu insertion loci (TPA25, D1, APO, PV92, FXIIIIB and ACE) from 18 endogamous caste and tribal populations (900 samples). The regions studied include North (5 populations), Central and South (9 populations) and Western India (4 populations). Overall spectrum of variation in these populations is very interesting at different geographical and cultural levels. High level of insertion frequencies was observed in some highly inbred groups. Average levels of heterozygosities were found to be relatively high in these populations (range 41% to 49.8%). The genetic diversity coefficient GST among this group of populations was observed to be high. Phylogenetic trees and principal components analysis (PCA) computed from Alu frequencies provide support for socio-cultural and geographical assignment of these populations in Indian population structure. Results are discussed with reference to population origins and human evolution in India.

Most Puerto Rican mtDNAs are of Native American origin and exhibit low genetic diversity. *J.C. Martínez-Cruzado¹, G. Toro-Labrador¹, J.A. Salas-Luciano¹, M. Estévez-Montero¹, M. Troche-Matos¹, E. Gómez-Sánchez¹, A. Román-Colón¹, M. Latorre-Estéves¹, L. Godoy-Muñoz¹, R. Rivera-Torres¹, A. Lobaina-Manzanet¹, H. Sánchez-Cruz¹, P. Ortiz-Bermúdez², F. Cruz-Guilloty¹, I.Y. Navarro-Millán¹, J.S. Ramírez¹.* 1) Department of Biology, University of Puerto Rico at Mayagüez, Mayagüez, PR. 00681; 2) Department of Bacteriology, Microbiology & Immunology, University of Wisconsin at Madison, Madison, WI 53706.

This is an ongoing program of research that aims to study the genetics of the extinct Taíno people that inhabited Puerto Rico at the time of the Spanish colonization, and to estimate the contribution of the people from sub-Saharan Africa, Europe, and the Americas to the mitochondrial gene pool of the highly admixed population of Puerto Rico. Through RFLP analysis of Native American mtDNAs collected mainly in western Puerto Rico, a highly structured distribution of the subhaplogroup frequencies has been found, suggesting that the contribution of Native Americans foreign to Puerto Rico is minimal. Haplogroup A covers 55% of the Native American mtDNAs, and subhaplogroups A2 and C1 make 41.4% and 34.5% of these, respectively. Despite of the fact that most of the linguistic, biological and cultural evidence suggests that the Ceramic culture of the Taínos originated in or close to the Yanomama territory in the Amazon, the high frequency of haplogroup A suggests that the Yanomami, who lack haplogroup A mtDNAs, are not the main ancestors of the Taínos. However, more detailed analysis of Puerto Rican haplogroup C mtDNAs is highly consistent with an origin within or close to the Yanomama. In addition, a sample selection procedure designed to provide a sample representative of the Puerto Rican population is being used to determine the maternal contribution of people from different continents to Puerto Ricans. Hair roots from 781 subjects have been collected in 28 municipalities in Puerto Rico with informed consent. A substantial Native American ancestry has been found. Of 115 samples analyzed to date, 71 (61.7%) have been identified as having Native American mtDNA, 18 (15.7%) sub-Saharan African, 5 (4.3%) Caucasian, and 21 (18.3%) need further analysis.

Gene mapping and admixture on the island of Kosrae. *D. Shmulewitz¹, S.C. Heath², T. Lehner¹, S.B. Auerbach³, E. Asilmaz¹, M.L. Blundell¹, L. Petukhova¹, J.D. Winick¹, P.C. Verlander¹, Z. Han¹, J.L. Breslow¹, J. Ott¹, M. Stoffel¹, J.M. Friedman¹.* 1) Starr Center Human Genetics, Rockefeller Univ, New York, NY; 2) Lab Mathematical Genetics, MSKCC, New York, NY; 3) HRSA, Dept Health & Human Services, New York, NY.

The Pacific island of Kosrae is well suited for genetic mapping of complex traits; it was originally settled by a small number of founders and the population has a high prevalence of several metabolic disorders. Whaling ships visited Kosrae in the 19th century, resulting in significant admixture among the mostly Caucasian whalers and native Micronesians. This admixture is expected to increase the genetic variance and lead to extensive linkage disequilibrium (LD), which could potentially facilitate genetic mapping. To test this, we genotyped Y, mitochondrial (mt), and autosomal markers on a set of Kosraean, Caucasian, and other Micronesian DNAs. Genotypes were scored using ABI's fluorescent fragment (Y and autosomal microsatellites) or sequence (mtDNA) analysis methods. To quantify the admixture, we used PHYLIP to cluster the Y and mt haplotypes, and calculated the percentage of Kosraean haplotypes that clustered among themselves or with the other populations. To measure the genetic variance, we used mt sequence to calculate nucleotide diversity and compared allele frequencies for autosomal markers to the CEPH frequencies. LD was measured using closely spaced markers on chromosome 1. The Y clustering suggests that 50% of the male lineages were Caucasian in origin. The extent of admixture in the female lineages, genetic variance, and LD on chromosome 1, are currently being evaluated. As it has been 5-10 generations since the admixture occurred, the regions of LD are expected to extend 5-75 cM. This should prove useful for identifying chromosomal segments derived from one or the other founder populations. If a region is Kosraean in origin, LD from the original Kosraean settlers, expected to be 0.5-5 cM in length, could be used to fine map the causal genes. These studies may ultimately be of general importance, and reveal the potential utility of admixed populations for the mapping of complex traits.

Validity of homozygosity test of selective neutrality at microsatellite loci. *H. Xu¹, A. Renwick², M. Kimmel², R. Deko³, R. Chakraborty¹.* 1) Human Genetics Center, University of Texas, Houston, TX; 2) Dept. Statistics, Rice University, Houston, TX; 3) Dept. Environmental Health, Univ. Cincinnati, Cincinnati, OH.

Distribution of homozygosity in a sample given the number of segregating alleles has been widely used as a test of selective neutrality for protein and immunological markers. The underlying theory is that the allele frequency spectrum observed in a sample for a fixed number of observed alleles is independent of all parameters of the allele frequency distribution. Thus, the critical region of the test procedure can be evaluated from the knowledge of sample size (n) and number of alleles (k). Analytical proof of this theory holds for a population of constant size under mutation-drift equilibrium with the Infinite Allele Model (IAM) of mutations. Simulation studies with unrestricted equiprobable mutations between a finite number of alleles indicate that the theory is approximately valid in a wider context. However, theoretical as well as empirical data demonstrate that microsatellite loci evolve with stepwise mutations (SMM) where the repeat size changes induced by single mutations are restricted (most often increase/decrease of one repeat unit). We present some results on the validity of homozygosity test of neutrality for loci evolving under such mutation models. Coalescence-based simulations indicate that the conditional distribution of allele frequency spectrum, given a number of alleles (k), is no longer independent of the SMM parameters. In fact, with increasing value of q (product of effective population size, N , and mutation rate, m), the distribution of sample homozygosity (F) shifts towards left (i.e., smaller values). As a consequence, published tables of critical regions may not be valid. Further, we show that population substructure causes a similar effect. These results imply that other features of allele/genotype frequency distributions at microsatellite loci may be needed to detect effects of natural selection. (Research supported by US Public Health Service Research grants GM 41399, GM 45816, and GM 58545 from the National Institutes of Health and a NSF graduate fellowship to AR).

Genetic susceptibility, systolic hypertension and the risk of poor cognitive function. *R. Peila, R. Havlik, L.J. Launer.* EDB, National Institute on Aging, Bethesda, MD.

Cognitive impairment is often an early symptom of dementia. The trait has a complex etiology, with multiple environmental and genetic risk factors. Untreated mid-life systolic blood pressure has been found inversely related to late-life cognitive functioning. Apolipoprotein E (APOE), in particular e4 allele, is an important genetic risk factor for poor cognitive function and Alzheimers disease (AD). We investigated whether mid-life high systolic blood pressure (HSBP) and APOE e4 interact to increase the risk of late-life poor cognitive function, and whether the use of hypertension medication modifies their effect. Data were obtained from a population-based cohort of 3605 elderly Japanese-American men, enrolled in the Honolulu-Asia Aging Study (HAAS). Systolic blood pressure (SBP) was measured during three exams (1965, 1968-1970, 1971-1974) when they were middle aged (age range 45-68 years at the first exam). In 1991-1993, cognitive function was evaluated with the 100-point Cognitive Abilities Screening Instrument (CASI). APOE genotype was characterized in 1991. To test the independent and joint effect of APOE e4 allele and mid-life HSBP (SBP³160mmHg), four categories were created: NoHSBP/ Noe4, used as the reference group; NoHSBP/e4; HSBP/Noe4; HSBP/e4. The analysis was stratified by ever use of hypertension treatment. Relative risk (RR, p-value) of poor cognitive function (CASI <74) relative to good cognitive function (CASI³82) was evaluated after adjusting for age, education, stroke, ankle-brachial index, coronary artery disease, smoking and alcohol use. In the untreated group, compared to the NoHSBP/ Noe4 subjects, the RR of poor cognitive function for NoHSBP/e4 was 1.27 (p=0.2), for HSBP/Noe4 it was 2.32 (p=0.2) and for HSBP/e4 it was 9.2 (p=0.04). In the treated group, the risk of poor cognitive function for those with both risk factors was 1.8 (p=0.22). The results suggest that hypertension has a particularly adverse effect on cognitive function in persons with higher genetic susceptibility. This effect may be modified by antihypertensive treatment.

Genetic evidence for an East Asian contribution to the second wave of migration to the new world. *B. Su^{1,2}, X. Song¹, Y. Ke¹, F. Zhang¹, J.T. Lell³, D.T. Wallace³, P.A. Underhill⁴, R.S. Wells⁵, D. Lu¹, R. Chakraborty², L. Jin^{1,2}.* 1) Institute of Genetics, and Morgan-Tan International Center for Life Sciences, Fudan University, Shanghai, China; 2) Human Genetics Center, Univ Texas Sch Pub Hlth, Houston, TX; 3) Center for Molecular Medicine, Emory University School of Medicine, Atlanta, GA; 4) Department of Genetics, Stanford University, Palo Alto, CA; 5) Wellcome Trust Center for Human Genetics, University of Oxford.

It is unclear whether East Asians were involved in the earliest migrations to the New World. In this study, more than 2,000 Y chromosomes from East Asia, Siberia, and America were analyzed using Y chromosome specific biallelic and microsatellite markers. Our results demonstrate that RPS4Y711T, an Asian specific allele, has a wide distribution in East Asia, and reaches the highest frequency in North Asian populations. The gene diversity of RPS4Y711Ts in East Asians, based on microsatellites, is five times higher compared to those in Siberians and American Indians suggesting a northward migration starting in East Asia. This northward migration is marked by a founder event in North Asian populations which are associated with the prevalence (88.8%) of a unique microsatellite allele, DYS391-9 in most of the North Asian RPS4Y711T Y chromosomes, which were originally derived from DYS391-10 alleles in East Asian populations. The founder effect was further reflected by restricted distribution of an A to G mutation at another Y chromosome locus (M48) in Siberian populations. Hence, we propose that a prehistoric migration associated with the RPS4Y polymorphism started from the southern part of East Asia, crossed mainland China, and reached Siberia about 15,000 years ago, which eventually contributed to the second migration wave to the New World.

***MC1R* gene variation in normally pigmented South African individuals.** *M. Ramsay, P.R. John.* Dept Human Genetics, SAIMR and University of the Witwatersrand, Johannesburg, South Africa.

Skin, hair and eye pigmentation is a polygenic multifactorial trait determined by the cumulative effects of multiple genetic variant alleles and environmental factors. *MC1R* is one of the genes involved in pigmentation, and has been implicated in the red hair and pale skin trait in human Caucasoid individuals. The present study was undertaken to identify variation at the *MC1R* locus in normally pigmented individuals in two African populations, sub-Saharan Negroids (22 unrelated individuals) and the San (17 unrelated individuals). The study showed considerable *MC1R* gene sequence variation with the detection of eight synonymous and three non-synonymous mutations. The three non-synonymous mutations had not previously been reported; one (L99I) was found in a single San individual, one (S47I) was detected in a single Negroid individual and the third (F196L) was detected in 5 Negroid individuals (5/44; 0.11). The functional significance the F196L mutation is not known. Of the eight synonymous mutations found, only three, L106L, F300F, and T314T (also known as A942G), had been reported previously. T314T was the only variant that showed a significant difference between the Negroid and San populations (0.477 and 0.059 respectively, $p=5.8 \times 10^{-5}$). The G allele was thought to be the ancestral type since it had previously been found in non-human primates. Its low frequency in the San may be the result of random genetic drift in a population of small size, or selection. In addition to these studies, 7 unrelated red-haired South African individuals of European origin were studied for *MC1R* mutations. Two *MC1R* mutations were detected in each individual, either in the homozygous or compound heterozygous state. Four novel mutations were identified, S83P, Y152X, A171N and P256S. The R151C and R160W variants were observed at frequencies of 0.286 (4/14) and 0.214 (3/14), respectively. In general, individuals with lighter skin have more non-synonymous *MC1R* mutations suggesting that this gene is likely to play a significant role in normal pigment variation.

HARDY-WEINBERG EQUILIBRIUM DIAGNOSTICS. *A. Rogatko, J. Babb, M. Slifker.* Biostatistics, Fox Chase Cancer Ctr, Philadelphia, PA.

We propose two diagnostics for the statistical assessment of Hardy-Weinberg equilibrium. One diagnostic is the posterior probability of the complement of the smallest highest posterior density credible region that includes points in the parameter space consistent with the hypothesis of equilibrium. The null hypothesis of equilibrium is to be rejected only if this probability is less than a pre-selected critical level. The second diagnostic is the proportion of the parameter space occupied by the highest posterior density credible region associated with the critical level. These Bayesian diagnostics can be interpreted as analogs of the classical type I and II error probabilities. They are broadly applicable: they can be computed for any hypothesis test, using samples of any size generated according to any distribution. Our paper includes the derivation of diagnostics, computational aspects, asymptotic properties, and examples of application for a single autosomal locus with multiple co-dominant alleles, and a single X-linked locus with two co-dominant alleles. A computer program - HWDIAG (available for Windows 98/NT) and user's guide can be downloaded from http://www.fccc.edu/users/rogatko/hwdiag_guide.html.

b GLOBIN HAPLOTYPE FREQUENCIES AND DISTRIBUTION IN SARDINIAN AND CORSICAN

POPULATIONS. *M.S Ristaldi*¹, *V. Latini*², *L. Vacca*², *L. Varesi*³, *M. Memmi*³, *F. Marongiu*¹, *G. Sole*¹, *G. Vona*². 1) IRTAM-CNR, Selargius, Cagliari, Italy; 2) Department of Experimental Biology, University of Cagliari, Italy; 3) Faculty of Sciences and Techniques, University of Corsica, France.

Sardinia and Corsica are the second and third largest islands in the Mediterranean basin and are only 12 Km apart from each other. The populations of the two islands are different from all the other Mediterranean populations. Sardinia is characterised by a great internal heterogeneity as well, which is mainly due to: 1) Internal geographical barriers. 2) Strict isolation and the accompanying great level of endogamy and inbreeding. 3) Past endemic malaria in selected areas of the island. Recent evidences suggest that internal microgeographic diversity may exist in Corsica as well. Microgeographic diversity due to isolation is of pivotal importance to distinguish between true genetic identity by descent and randomly occurring identity. This study has been designed to examine the genetic constitution of the populations from the two islands as a prerequisite for starting in Corsican and Sardinian villages the mapping of genes involved in complex diseases. As a measure of the genetic diversity between and within the two islands we analysed the distribution of the b-globin haplotypes in the populations of some Sardinian and Corsican villages. A total of 536 chromosomes have been analysed. In each chromosome 9 polymorphic restriction sites mapping b-globin gene cluster were analysed. The minimum sample size per village was of 24 individuals (48 chromosomes). Since selection by malaria could account for the different haplotypes distribution we carried out a screening for the most common b-thal mutations to exclude from the sample haplotypes containing b-thal mutations. Individuals have been selected only when both grandparents belonged to the village populations. Differences in the distribution of the b-globin cluster haplotypes were detected in the normal populations from the different village examined. These preliminary data confirm the existence of internal microgeographic diversity within the two islands.

Distribution of CCR2 and CCR5 alleles in children with respiratory disorders. *V. Romano-Spica, A. Ianni, D. Arzani, S. Majore.* Institute of Hygiene (Dir. Prof. G.C. Vanini), Catholic University, Rome, Italy.

Respiratory tract illness represents a major health problem in childhood, for the high incidence and possible consequences on lung function. Recurrent lower respiratory infections and asthma in 1999 showed a 7.9% and 9% frequency in Italy, respectively. Although some environmental risk factors have been associated to respiratory disease, knowledge about genetic predisposition is still limited. Promising perspectives are being disclosed by polymorphism analysis at genes responsible for the immune system regulation. Chemokine receptors (CCR) are involved in host inflammatory response against invading pathogens. A 32-bp deletion in the CCR5 gene (CCR5D32) and a G-to-A point mutation in the CCR2 gene (CCR264I) have been first associated to resistance to HIV-1 infection and recently involved in several immuno-related disorders. We evaluated the distribution of CCR2 and CCR5 allelic forms in children with low tract respiratory disease, following strict clinical case-selection. A total of 182 genomic DNA samples were analyzed, including 33 children with recurrent lower respiratory infections, 19 children suffering from asthma and 130 controls randomly selected from the general population. Genotype frequency was determined after electrophoresis or restriction analysis of the amplified products. CCR264I mutant allele showed a frequency of 0.2 for cases with recurrent infections, 0.24 for cases with asthma, and 0.07 for controls, with a statistically significant difference between each group of cases and controls ($p=0.002$ and 0.007 , respectively). CCR5D32 allelic frequency was 0.03 for cases with recurrent infections, 0.105 for cases with asthma, and 0.04 for controls. CCR5 allelic counts did not show a statistically significant difference between cases and controls. Both CCR2 and CCR5 genotype distribution was in equilibrium as predicted by the Hardy-Weinberg equation. These preliminary findings suggest a possible role for CCR-family genes in determining individual susceptibility to childhood respiratory disorders.

Genetic contribution of mtDNA and Y chromosome Amerindian haplotypes, to aboriginal and mixed Chilean populations. *M.P. Carvalho¹, F. Rivera¹, C. Morales¹, P. Rocco¹, M. Moraga², J.F. Miquel³, F. Nervi³, F.*

Rothhammer². 1) Depto. Biología Celular y Molecular, Fac. Ciencias Biológicas, P. Universidad Católica de Chile,; 2) Programa de Genética Humana, ICBM, Fac. Medicina, Universidad de Chile,; 3) Depto. Gastroenterología, Fac. Medicina, P. Universidad Católica de Chile, Santiago, CHILE.

The analysis of mtDNA and Y chromosome polymorphisms have been extensively used to study migrational events, and also maternal and paternal genetic contribution on populations. We analyzed three aboriginal Chilean populations, Aymara, Pehuenche and Mapuche, and a mixed population from Santiago. The frequencies of mtDNA haplogroups A,B,C and D, were determined for 336 aboriginal individuals, and 162 people from Santiago. The Y chromosome loci analyzed were: DYS 287 (Alu insertion), two SNP loci, DYS 199 and DYS 271, DYS 19 (STR) and the alphoid system (ah). The most frequent Amerindian haplotype characterized is: DYS 287(-)/DYS 199T/DYS 271A/DYS 19A,B/ah II,I. The presence of this Amerindian haplotype was analyzed in 162 aboriginal individuals and 45 people from Santiago. Chilean aboriginal groups revealed a variable frequency distribution of mtDNA haplogroups from north to south. Haplogroup B is the most frequent in the North (Aymaras), 57%, while haplogroups C and D are the most frequent in the two southern populations. In the 3 aboriginal groups 100% of individuals show one of the Amerindian mtDNA haplotypes. In the mixed population from Santiago all 4 Amerindian haplogroups showed a frequency of 84% being C and D the most frequent. Regarding Y chromosome contribution to aboriginal populations we found a 65% of Amerindian haplotypes, 17% of Caucasoid or European and 7% of African or Caucasoid. Interestingly the population from Santiago revealed only a 23% of the Amerindian Y chromosome haplotype. These results are in agreement with the history of Spanish colonization of Chile, during the 16th and 17th centuries, in which an admixture of Spanish men with Amerindian women occurred. Also we agree with previous studies that the described haplotype is the most frequent among Amerindian groups. (Fondecyt 198-1111).

Genotype Determines the Effect of Cigarette Smoking on Pulmonary Function. *P.V. Tishler¹, V.J. Carey², T. Reed³, R.R. Fabsitz⁴.* 1) VA Medical Ctr, Harvard Medical School, West Roxbury, MA; 2) Channing Lab., Brigham & Women's Hospital, Harvard Medical School, Boston, MA; 3) Med. Genetics, Indiana Univ. School of Medicine, Indianapolis, IN; 4) NHLBI, NIH, Bethesda, MD.

The effect of cigarette smoking on pulmonary function is highly variable. Some heavy smokers retain near normal pulmonary function while others are profoundly impaired. The role of genotype in this process is unknown. In order to decompose longitudinally evolving phenotypic variation into effects of genotype and exposure, both of which may vary with time, we developed a model for analysis of data from repeatedly observed twinships. Using maximum likelihood estimation in a highly flexible correlation model, we tested heritability hypotheses regarding the dependence of quantitative twin-twin phenotypic similarity on genotype in an environment of continuously varying twin-twin exposure. We applied this model to 2 chronologic data sets (including smoking history and forced expiratory volume in one second - FEV1) from 376 twinships enrolled in the NHLBI Male Veterans Twin Study. Strong heritability of FEV1 was confirmed ($p < .01$). Similarity in change over time in residual FEV1 between twins with a concordant smoking history was more pronounced for MZ twins ($r = 0.71$) than for DZ twins ($r = 0.34$). Similarity in change over time in residual FEV1 declined with discordant smoke exposure. Even in those twins differing in smoking history by ³25 pack years, however, the MZ twin correlation (r) for change in residual FEV1 was 0.54; that for DZ twins was 0.16. The smoking concordance-dependent correlation functions for MZ and DZ twins were significantly different ($p < .001$). We conclude that the effect of cigarette smoking on pulmonary function is controlled by genetic factors, albeit with appreciable modulation by extent of smoke exposure.

Single nucleotide polymorphisms (SNPs) in alcohol dehydrogenases and risk of oral clefts in humans: different effects of maternal and child genotypes. *S.R. Diehl¹, M.H. Khoshnevisan¹, T. Wu¹, C. Sun¹, M. Mazeheri², R. Long², A. Miller Chisholm¹.* 1) Craniofacial Epidemiology and Genetics Branch, NIDCR, NIH, Bethesda, MD; 2) Lancaster Cleft Palate Clinic, Lancaster, PA.

Nonsyndromic cleft lip and palate (CL/P) has high heritability, but multiple susceptibility genes and environmental interactions underlie its etiology. Since this birth defect occurs during the first trimester of pregnancy, both the mother's and the child's genotypes may affect disease risk, further complicating analyses of candidate gene SNP associations. We conducted a family-based association study of SNPs in alcohol dehydrogenase (ADH) genes and maternal alcohol consumption during pregnancy. We used a recently developed statistical approach, which assesses effects of the maternal and affected child's genotypes on disease risk. Our sample included 163 families with ³¹ CL/P-affected member and 448 subjects with DNA available for genotyping. Two SNPs in ADH2 and one in ADH3 were genotyped by PCR-RFLP or OLA methods. We found associations in opposite directions for mothers' and affected children's genotypes for two SNPs. For example, risk was increased in children having the ADH3 "12" genotype, but decreased in mothers with this same genotype. We also found a significantly elevated frequency of the slow metabolizing "12" and "22" genotypes of the ADH3 SNP in CL/P-affected children whose mothers consumed alcohol during the first trimester compared to affected children whose mothers didn't drink. This suggests a gene by environment interaction: CL/P risk associated with the child's genotype at this SNP depends on maternal alcohol exposure. These examples illustrate some of the issues that must be incorporated into SNP linkage disequilibrium studies of complex diseases.

Parent-child transmission of intermediate *FMR1* CGG repeat alleles: Examination of factors associated with repeat instability. A.K. Sullivan, D.C. Crawford, K.L. Meadows, B. Wilson, S.L. Sherman. Dept. of Genetics, Emory University School of Medicine, Atlanta, GA.

Fragile X syndrome results from the expansion of a CGG trinucleotide repeat located in the 5' UTR of the *FMR1* gene. Unaffected individuals have stable alleles with ~6-40 repeats, premutation carriers have unstable alleles with ~60-199 repeats and affected individuals have >200 repeats. "Intermediate" alleles, ranging from 40-60 repeats, lie in the gray zone; that is, some alleles are unstable while others are stable. The ability to identify intermediate alleles that are susceptible to expansion is important for genetic counseling of such carriers. Several factors have been suggested to influence CGG instability such as size and purity of the repeat, haplotype background and sex of transmitting parent. To better characterize risk factors for instability among intermediate alleles, we have examined 202 parent-child transmissions of intermediate alleles found in 86 families. Out of the 158 transmissions with 40-49 repeats, 5 (3.2%) were unstable, 3 of which had parental alleles of 47-49 repeats. Out of the 44 transmissions with 50-60 repeats, 10 (22.7%) were unstable, 7 of which had parental alleles of 55-61 repeats. Most unstable transmissions were expansions (12/15) and most (10/15) were paternal transmissions. Of the 3 contractions identified, 2 were paternal transmissions. We are currently determining both repeat structure and haplotype backgrounds for all intermediate alleles. Initial sequencing results indicate that risk factors, in addition to the purity of the 3' end of the repeat, are important to the susceptibility of instability.

Friedreich Ataxia Expansion Haplotypes in Acadians. *D.J. Ragusa, D. Zhining, B.J. Keats.* Dept Biometry & Genetics, Louisiana State Univ Hlth Sci, New Orleans, LA.

Friedreich ataxia (FRDA) is the most common of the hereditary ataxias, occurring with a frequency of approximately 1 in 50,000. This autosomal recessive neurodegenerative disorder is characterized by progressive gait and limb ataxia, dysarthria, and age of onset around puberty. The majority of FRDA patients are homozygous for a GAA repeat expansion within the first intron of the frataxin gene on chromosome 9q13. Comparative studies of the yeast frataxin homologue suggest that it is associated with the maintenance of physiological Fe²⁺ levels within mitochondria. Acadian FRDA patients tend to have later age of onset, slower rate of progression, and older age at death than typical FRDA patients, although the mean and variance of the expansion repeat size are about the same. Using five polymorphic markers (FAD1, ITR3, F5225, ITR4, CS2) within or near the frataxin gene, we determined the haplotypes for 98 Acadian chromosomes with the GAA repeat expansion. Our results reveal haplotypes that differ in frequency from those reported for non-Acadian chromosomes with the expansion. The most common non-Acadian expansion haplotype is AT2CC with a frequency of 51%. Two others, AT3CC and AT2CT, are relatively common with frequencies of 21% and 14%, respectively. These three haplotypes are found for only 3.6% of chromosomes with normal sized alleles. In contrast, we did not find the AT[2/3]CC haplotypes in the sample of Acadian chromosomes with the expansion, but the haplotype for more than 95% is AT[2/3]CT. Our results suggest that most of the GAA repeat expansions in the Acadian population are derived from a single founder chromosome that is relatively rare among non-Acadian affected individuals. This finding may be part of the explanation for the less severe phenotype in FRDA patients of Acadian ancestry.

Modelling population admixture in a case-control design. *J.J. Hottenga^{1,2}, J.J. Houwing-Duistermaat¹, T.H. Stijnen¹, C.M. van Duijn¹.* 1) Epidemiology & Biostatistics, Erasmus University, Rotterdam, Zuid Holland, The Netherlands; 2) MGC - Department of Human and Clinical Genetics, Leiden University, Leiden, Zuid Holland, The Netherlands.

Association study or linkage disequilibrium mapping is a powerful tool for the fine mapping of disease genes. Allelic association may be caused by a disease gene located near the marker locus. However allelic association may also be found if the studied population is a mixture of sub-populations with different genetic background and disease prevalence. We propose to model admixture using the information from a number of markers outside candidate regions. The unknown population status can be considered as missing data and the model can easily be fitted using the EM algorithm. The performance of the model was studied for various simulated data sets. The power to detect admixture was compared to other tests currently available as for example testing Hardy Weinberg equilibrium and the test proposed by Pritchard et al.

Diagnostic evaluation and counseling of 97 families with hearing loss. *L.D. Bason¹, U.K. Shah², W.P. Potsic², T. Dudley³, K. Lewis³, I.D. Krantz¹.* 1) Division of Human Genetics; 2) Division of Otolaryngology; 3) Department of Audiology, The Children's Hospital of Philadelphia, The University of Pennsylvania School of Medicine, Philadelphia, PA.

Hearing loss is one of the most common birth differences affecting approximately 1/500 children. It is thought that greater than half of prelingual hearing loss is genetic in etiology. Hearing loss is a heterogeneous group of disorders, however the identification of multiple genes in which mutations result in hearing loss, makes molecular testing an important tool for establishing etiology. The combination of clinical genetics evaluation with molecular testing can offer a specific etiology for hearing loss in a substantial number of families and provides for more accurate genetic counseling and medical management. We report a summary of our experience in the Genetics of Hearing Loss Clinic at the Children's Hospital of Philadelphia since January 1999. Ninety-seven individuals or families with hearing loss have been evaluated through this clinic. Four were found to be due to non-genetic causes (intrauterine infection, graft versus host disease); 51 had isolated bilateral sensorineural hearing loss (SNHL) which was familial in 11 cases; 18 had unilateral SNHL including one familial case; 1 had bilateral conductive hearing loss; 23 had SNHL associated with additional findings (syndromic diagnoses were established in 8 of these cases). Connexin 26 (Cx26) screening was completed in 44 families and revealed positive results in 9 families. The group tested for Cx26 mutations was heterogeneous, with the majority being single affected individuals with a negative family history. As more hospitals implement newborn screening, an increasing number of children with hearing loss will be identified at a younger age. Early detection of hearing loss and establishment of its cause is beneficial to the child's medical and educational management and allows for accurate genetic counseling prior to the next pregnancy.

The M34T allele variant of Connexin 26. *R.A. Cucci¹, S. Prasad¹, P.M. Kelley², G.E. Green¹, K. Storm³, S. Willocx³, E.S. Cohn², G. Van Camp³, R.J.H. Smith¹.* 1) Department of Otolaryngology- Head and Neck Surgery, Iowa City, IA; 2) Boys Town National Research Hospital, Omaha, NE; 3) Department of Medical Genetics, Antwerp, Belgium.

GJB2 encodes the protein Connexin 26, one of the building blocks of gap junctions. Each Connexin 26 molecule can oligomerize with five other connexins to form a connexon; two connexons, in turn, can form a gap junction. Because mutations in GJB2 are responsible for approximately half of all congenital severe-to-profound autosomal recessive non-syndromic hearing loss (ARNSHL), the effect of Connexin 26 allele variants on this dynamic 'construction' process and the function of any gap junctions that do form is particularly germane. One of the more controversial allele variants, M34T, has been hypothesized to cause autosomal dominant non-syndromic hearing loss. In a screen of 545 families, we identified 23 persons from 9 families with the M34T allele variant. We present clinical and genotypic data that refutes this hypothesis and suggests that the effect of the M34T allele variant is dependent on the presence of specific mutation on the opposing allele.

Relative prevalence of different Cx26 variants and detection of new mutations in the Italian population. A.

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As part of the program of clinical and molecular characterization of different forms of hereditary hearing loss that we are conducting, the Cx26/GJB2 gene has been analyzed in almost 400 unrelated subjects affected by congenital or preverbal hearing impairment of various degree referred to our research and diagnostic center mainly from central-north of Italy. Among the Cx26 variants detected by PCR-SSCP analysis and direct sequencing, the 35delG mutation has been found on 65% of the mutated alleles. Several other non-35delG mutations have been identified, the second most common one in our population being E47X, that accounts for about 3.6% of the alleles, then the splice-site alteration IVS1+1G>A (2.5%), and the coding sequence mutations: R184P (2.5%), M34T (2.5%), L90P (1.6%), 167delT (1.5%), 310del14 (1.2%), 31del14 (0.8%), E147K (0.8%), delE120 (0.8%), G160S (0.4%), V153I (0.4%), R127C (0.4%). Five Cx26 new variants have been identified: 290_291insA and V193I, detected in hearing impaired individuals compound heterozygote for the 35delG mutation, and the substitution of the arginine at position 184 with a tryptophan (R184W) or with a glutamine (R184Q), as the sole mutation in deaf individuals. Finally, a "de-novo" substitution of the highly conserved arginine in position 75 with a Glutamine (R75Q) has been found, again as the only mutation, in a profoundly deaf child. This latter mutation alters a functionally very important residue, site of a heterozygous non-conservative aminoacid change with documented dominant negative function. The mutation we have found could also represent a dominant Cx26 variant. This work is supported by: Ricerca Sanitaria Finalizzata Regione Veneto .N 878/03/98.

Connexin(Cx) Testing in a Nationwide Repository of Samples from Deaf Probands: Relevance to Clinical Practice. *A. Pandya*¹, *K. Oelrich*², *K.S. Arnos*², *R.J. Morell*³, *X-J. Xia*¹, *J. Albertorio*², *X.Z. Xiu*¹, *S.H. Blanton*¹, *T.B. Friedman*³, *W.E. Nance*¹. 1) Medical College of Virginia at Virginia Commonwealth University, Richmond, VA; 2) Gallaudet University, Washington DC; 3) NIDCD, Rockville, MD.

Mutations of Cx-26, account for 20%-30% of prelingual & 50%- 80% of recessive nonsyndromic deafness. 30delG accounts for 70% of the pathologic alleles, but more than 40 mutations have been reported. These findings have raised several practical issues in the interpretation of test results. For example heterozygous sequence variation in a deaf proband can be of uncertain significance. The observation that other Cx genes also cause deafness has raised the possibility of a digenic etiology in some cases. Some Cx-26 alleles appear to cause a dominant phenotype. In order to address these issues, we screened 260 probands for mutations in the Cx-26, 30 & 31 genes. The samples were derived from a Nationwide Repository of DNA from deaf multiplex and simplex probands. Of these 23.5% were Cx-26 positive, 85% of whom were homozygous for the 30delG mutation. Three novel mutations were observed, two reported previously and an I81M missense substitution. In 22(8.5%) heterozygous probands no changes in either the Cx-30 or 31 genes were found. The W44C mutation was observed in one family with dominant deafness. One deaf proband was homozygous for the polymorphic M34T change, the clinical significance of which is unclear. Audiometric data on 187 probands revealed variability in the severity of hearing loss. A heterozygous deletion of a single guanine residue at codon 21 in the Cx-30 gene observed in only one family suggests a low frequency of Cx-30 in contrast to Cx-26 mutations. Our studies confirm the existence of dominant transmission of Cx-26 deafness and provide little evidence for epistasis with other Connexin genes as a mechanism of hearing loss. Studies in large populations such as this will facilitate the appropriate interpretation of molecular test results to improve counseling, and support the development of clinical guidelines for the management of children with deafness.

Frequency of Connexin 26 (GJB2) mutation carriers in the Turkish population. *M. Tekin^{1,2}, N. Akar², S. Cin², S.H. Blanton¹, W.E. Nance¹, A. Pandya¹.* 1) Dept Human Genetics, Virginia Commonwealth Univ, Richmond, VA; 2) Dept Pediatrics, Ankara Univ School of Medicine, Ankara, Turkey.

Mutations in Connexin 26 (GJB2) gene are responsible for more than half of the cases with prelingual non-syndromic recessive deafness in many Caucasian populations. A single variant, 35delG, accounts for up to 70% of all pathologic Connexin 26 mutations in these countries. The carrier frequency of this mutation has been reported to range between 2.2% to 4% in Southern European populations. The carrier frequency of a second mutation, 167delT, has been reported to be 2.7 - 4% in Ashkenazi Jews. To evaluate the importance of these two mutations in the Turkish population we first screened three families with recessive non-syndromic deafness using PCR-RFLP based protocols. In all three families the 35delG mutation was demonstrated as the cause of deafness. We subsequently screened 674 hearing Turkish individuals whose DNA samples had been obtained for unrelated research studies at Ankara University School of Medicine. Heterozygous 35delG and 167delT mutations were detected in 12 (1.78%; 95% confidence interval 0.9% - 3%) and 2 individuals (in 632 samples) (0.31%; 95% confidence interval 0.1% - 1.1%), respectively yielding a combined gene frequency of 0.0105. Genotyping of our samples with closely linked polymorphic markers (D13S1236, D13S1275, D13S175, D13S141, D13S250) is in progress. These results provide an estimate of the carrier frequency of 35delG in Turkey and establish it as an important cause of non-syndromic deafness. Surprisingly the 167delT mutation, described to date only in Ashkenazi Jews, is also present in the Turkish population, although at a much lower frequency. Assuming random mating, the expected incidence of Cx 26 deafness would be about 11.1 per 100,000 births. However, the prevalence may be higher because of assortative mating.

Preponderance of MTHFR homozygosity among the Louisiana Hispanic newborn population. *T.F. Thurmon, J.A. Bocchini, Jr, K. Yanamandra, H. Chen, S. Ursin, D. Napper.* Dept Pediatrics-Genetics, Louisiana State Univ, Shreveport, LA.

We have begun several epidemiological studies as part of our March of Dimes birth defects foundation and LSU foundation grants in various genetic conditions such as medium chain acylcoA dehydrogenase (MCAD) deficiency, inherited thrombophilia including factor V Leiden, prothrombin II mutation, and the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR). We also have been studying the relationship of MTHFR genotypes and high risk pregnancies for NTD and chromosomal defects in our prenatal screening program. Data on MCAD deficiency and factor V Leiden in our newborn population have been presented in the previous ASHG meetings. Now we are presenting the data on our newborn population on the prevalence of C677T MTHFR mutation. We have studied the mutation frequency on about 2000 newborns. The overall mutant MTHFR gene frequency was 15.7% and normal MTHFR gene frequency was 84.3%. The carrier frequency and homozygous mutant MTHFR frequencies were 22.7% and 4.3% respectively in our newborn population. MTHFR homozygosity in our newborn population was 1.4%, 25%, and 13% respectively in our African-Americans, Hispanics and European-American newborns. Our data revealed the homozygosity in our Hispanics was twice that was found in European-Americans. Data and statistics in various ethnic groups will be presented.

A flexible multiplexed flow cytometry-based assay for HLA-DR b typing. *J.M. Baisch, E. Anguiano.* Genetics Laboratory, GeneScreen, Inc, Dallas, TX.

Low, intermediate, and high resolution HLA-DRB typing is performed using a range of techniques, including SSP, SSOP, and SBT. These technologies have limitations related to throughput, equipment needs, technical expertise, level of resolution, and cost. As such, laboratories have had to employ two or more technologies, sometimes in combination, if they require the capability to perform HLA-DRB testing at various levels of resolution, on a range of sample numbers, with short turnaround times. In an effort to provide a single assay that will afford laboratories these capabilities, we have employed a flow cytometry based technology (Luminex Corporation, Austin, Texas) to develop a flexible multiplexed method for HLA-DRB typing. This method uses microspheres that can be classified based on unique internal fluorescence characteristics. By attaching a DNA probe that detects specific HLA-DRB polymorphisms to a particular class of microspheres, the subsequent binding of labeled target to a specific set of microspheres, and accordingly to a specific set of probes, can be measured using a Luminex-100 instrument. Utilizing this technology, we have demonstrated that samples can be typed for HLA-DRB at an intermediate level of resolution using a multiplexed set of 39 microspheres. What's more, using the same assay, higher levels of resolution can be achieved by multiplexing the amplification reactions to include certain allele-specific amplifications and adding specific probes to the multiplex of microspheres, since the Luminex-100 is capable of monitoring up to 100 different classes of microspheres simultaneously. Multiplexed flow analysis using microspheres offers a flexible and cost-effective alternative for HLA-DRB testing. In addition, this approach offers the advantage of rapid processing and analysis time for HLA-DRB typing at any level of resolution or sample volume and can easily be expanded to test other HLA loci.

Distribution and phenotypic associations of mutations in patients with demyelinating neuropathies. C.F.

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Demyelinating and dysmyelinating diseases are among the most common inherited disorders of the peripheral nervous system. These diseases include Charcot-Marie-Tooth disease type 1 (CMT1), congenital hypomyelinating neuropathy (CHN), and Dejerine-Sottas syndrome (DSS). In a cohort of 119 patients referred with a demyelinating neuropathy prior to the availability of clinical genetic testing, 60% had a duplication of *PMP22*, 6% *GJB1* mutations, 5% *PMP22* mutations, 4% *MPZ* mutations, and 1% *EGR2* mutations. In addition, screening of 193 cases of familial or sporadic peripheral neuropathy identified several novel mutations in *PMP22*, *MPZ* and *SOX10* as well as a novel non-disease-associated mutation in *EGR2*. Novel *PMP22* mutations identified: Trp28Arg associated with CMT1 and deafness, His34Gln associated with Hereditary Motor and Sensory Neuropathy type V, and Leu71Pro associated with CMT1. Novel *MPZ* mutations identified: Gly123Cys associated with DSS, Val136Glu associated with CMT1, and Ile89Asn, Val92Met, Ile162Arg associated with CMT1. The *SOX10* mutation identified: Gln250STP associated with CHN and Waardenburg syndrome features. The novel non-disease-associated *EGR2* mutation identified: Gly451Val. We also report confirmation of several previously reported mutations and the results of screening several candidate genes.

DHPLC analysis of HNPCC: a rapid sensitive exon screen of hMLH1 and hMSH2. *J.F. Harvey¹, S.P. Haynes¹, D.M. Eccles².* 1) Wessex Regional Genetics Lab, Salisbury District Hospital, Salisbury, Wiltshire, United Kingdom; 2) Wessex Clinical Genetics Service, Level G, Princess Anne Hospital, Coxford Road, Southampton, Hants, SO16 5YA, United Kingdom.

hMLH1 and hMSH2 are two of the four mismatch repair genes mutated in hereditary non-polyposis colorectal cancer (HNPCC) and account for two-thirds of affected families. They have complex genomic structures (19 and 16 exons respectively) with diverse mutational spectra and few common mutations. We have developed a rapid highly sensitive screen of coding exons and splice junctions using a 96 well microtitre plate format with multichannel pipette transfer and intron derived primers. Mutations are detected using denaturing high performance liquid chromatography at two melt temperatures (derived using Transgenomic Wavemaker software) for PCR fragments amplified with AmpliTaq Gold. We identified all 29 unique, mutations/ polymorphisms (14 amino acid changes, 2 splice site mutations, 2 insertions and 11 deletions) previously found in our family samples by combined SSCP/heteroduplex analysis on silver stained polyacrylamide gels. The resolution of DHPLC heteroduplex analysis suggests that sensitivity of this approach is well in excess of the original gel based method.

Mammography behavior after receiving a negative *BRCA1* mutation test result in the Ashkenazim: A community-based study. *S.E. Plon, L.E. Peterson, C.S. Richards, L.C. Friedman.* Baylor College of Medicine, Houston, TX.

Background: Women who test negative for mutations in breast cancer susceptibility genes remain at risk of developing breast cancer. Therefore, it is important to define the impact of a negative test result on subsequent cancer screening behaviors. Studying the impact of DNA testing on the Ashkenazim is especially relevant because the frequency of these founder mutations and the lower cost of DNA testing have facilitated testing in this population.

Methods: We followed a community-based sample of 309 Ashkenazi Jews with or without personal or family histories of breast cancer who were offered testing for the 185delAG *BRCA1* mutation in 1996. Of 309 participants, 193 were female, mutation negative, average risk (based on family history of cancer), and unaffected with breast cancer at baseline. 118 women provided complete data on self-reports of mammography behavior, perceived risk and worry about breast cancer at baseline and on Year One and Year Two follow-up questionnaires.

Results: Average risk women age 50 and older had 91.7% compliance with mammography for the year prior to entry (baseline). There was no significant change ($p=0.775$) in mammography at the Year One (88.3%) and Year Two (91.7%) follow-up. There were significant decreases in breast cancer worry ($p=0.002$) and perceived risk ($p=0.027$). Women under age 50 demonstrated an increase in mammography (49.2% at baseline, 62.7% at Year One and 67.1% at Year Two, $p=0.035$) with decreases in worry ($p=0.002$) and perceived risk ($p=0.012$). Logistic regression analysis showed that age, physician recommendation, worry, and perceived risk all were significantly related to having a mammogram at Year Two with an odds ratio greater than one for older age, physician recommendation and increased perceived risk but significantly less than one for increased breast cancer worry. Six women were diagnosed with breast cancer during the two years subsequent to testing.

Conclusion: Receipt of negative *BRCA1* test results in a cohort of Ashkenazi Jewish women did not have a negative impact on mammography behavior two years after genetic testing.

MOLECULAR GENOTYPING OF HEMOPHILIA A: EXPERIENCE OF A CLINICAL TESTING

LABORATORY. *M.P Citron, S.N Palmer, S. Verma, L. Godmilow, T. Ganguly, A. Ganguly.* Genetic Diagnostic Laboratory, Department of Genetics, University of Pennsylvania, Philadelphia, PA.

Clinically feasible molecular genotyping of Factor VIII gene is a technological challenge as the gene is large. At the same time every clinical test requires a rapid turnaround time as well as high sensitivity and specificity. We have developed a comprehensive genotyping scheme for Factor VIII gene with a fast turn around time and high sensitivity. This study reports the spectrum of mutations observed in 108 consecutive Hemophilia A patients following molecular genotyping of Factor VIII gene. Genotyping includes Southern blot analysis to detect the common inversion of intron 22 and direct molecular analysis of all coding exons of Factor VIII gene. Of 78 male hemophiliacs tested, mutations were detected in 68(87%) individuals (18 inversion, 9 nonsense, 17 missense, 12 frameshift, 5 splice junction mutation and 7 rearrangements leading to insertions or deletions of the factor VIII gene). In addition, 30 females were tested for mutation carrier status due to their family history. Mutations were found in 21(70%) female individuals (14 inversion, 4 missense, 2 frameshift and 1 splice junction mutation). The spectrum of point mutations detected include a set of 37 novel mutations. The relative positions of these mutations and the correlation with the severity of the disease will be presented. Six males with Factor VIII level less than 1% did not reveal any mutation in the coding sequence. Also, 9 females including 2 hemophiliacs and 4 obligate carriers were negative for any coding sequence mutations. Assuming the diagnosis of hemophilia A to be correct, we hypothesized that the mutations are either masked by the wild type allele, or are present in non-coding regulatory regions. In order unmask the hidden mutations, we have modified the southern blot approach for fine mapping of aberrant restriction fragments. This has led to the identification of 2 new mutations in 3 males and 3 mutations in 6 females tested. The sensitivity of detection is thus greater than 90%. The intensified searches have made a significant impact on decisive medical management of the disease.

MOLECULAR STUDY OF JUVENILE NEPHRONOPHTHISIS IN TUNISIA. *R. M'RAD¹, A. KAMMOUN², F. BEN MOUSSA³, F. MAAZOU¹, R. GOUCHA³, L. BEN JEMAA¹, N. SMAOUI¹, H. BEN MAIZ³, H. CHAABOUNI¹.* 1) genetic departement , charles nicolle hospital, tunis, tunisia; 2) paediatric departement, charles nicolle hospital, tunis, tunisia; 3) nephrology departement,charles nicolle hospital, tunis, tunisia.

Nephronophthisis is an autosomal recessive kidney disease, characterised by cyst formation in cortico-medullar junction and a sclerosing tubulo-interstitial nephropathy. The disease is usually pauci-symptomatic in an early phase but invariably evolves end stage renal failure in childhood or early adulthood. Juvenile nephronophthisis (NPH1) is the most common genetic cause of renal failure in children, maps to chromosome 2q12-q13. The responsible gene has been identified and encodes for nephrocystin. An NPH2 locus has been assigned by linkage studies to chromosome 3q. The discovery of the NPH1 gene has prompted research into putative genotype phenotype correlations. We screened by PCR 49 Tunisian patients with a clinical features suggesting NPH diagnosis. We found a large homozygous deletion at NPH1 gene involving nephrocystin in 10 cases. No cases with tapeto-renal degeneration was associated with deletion of the nephrocystin. The remaining 39 patients had no apparent homozygous molecular deletion of nephrocystin. Some of them may have heterozygous deletion associated with point mutation. In spite of this genetic heterogeneity the two groups with and without detectable molecular defect of nephrocystin showed similar renal defects and comparable cumulative survival considering the start of dialysis as an end point. These data show that a molecular defect of nephrocystin is involved in 20% of Tunisian patient with clinical features suggesting NPH diagnosis. Detection of NPH1 deletions by PCR represents a simple non-invasive method for precise diagnosis in patients suspected of having NPH.

Mitotic index in skin fibroblast and short-term whole blood cultures as a biomarker for Alzheimer's disease in adults with and without Down Syndrome. *E.C. Jenkins, L.L. Ye, S.-Y. Li, W.B. Zigman, N. Schupf, C.M. Miezczeski, S. Sklower Brooks, W.P. Silverman.* New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY.

Currently, there are no biomarkers that can be employed in routine clinical practice to confirm the presence of Alzheimer's disease (AD). Development of a reliable and valid routine test for AD would facilitate early diagnosis and treatment, as well as research focusing on etiology and characterization of disease onset. Previously, we have reported finding reductions in mitotic indices in skin fibroblast cultures from five individuals with AD (NeuroReport 1998;9:3587-3861). We now report that 100% of a group of 13 individuals with AD (i.e., mostly familial cases ranging in age from 47 to 92 years) exhibited fewer mitotic figures in culture than did paired age- and sex-matched control individuals without AD ($p < .001$). We also found reduced mitotic indices in short-term whole blood cultures from individuals with [karyo- and apo-E typed] Down syndrome (DS), ranging in age from 47-66 years, who have been diagnosed as having dementia (presumably due to AD) compared to a group of age- and sex-matched nondemented individuals with DS. In these cases, the presence of dementia was established based on an objectively documented history of progressive memory loss, disorientation, and functional decline over a period of at least one year, and a diagnosis of probable AD by their own clinicians. All specimens were cultured in 199 (GIBCO) and the slides were coded and read blind to clinical status. Using our mitotic index findings, we classified individuals correctly in 14 of 18 cases, with 2 false-positive and 2 false-negative decisions. These results suggest that mitotic index in both skin fibroblast and short-term whole blood cultures should be further investigated as a diagnostic indicator for AD. {This work was supported in part by New York State Office of Mental Retardation and Developmental Disabilities, Alzheimer's Association Grant IIRG-99-1598, and by National Institutes of Health grants PO1 HD3587-13, HD37425-02, and RO1 AG014673-02.}.

Spinal Muscular Atrophy: Implications for Diagnostics and Pathogenesis. *M.D. Mailman¹, A.C. Papp¹, P.J. Snyder¹, J.W. Heinz¹, M. Sedra¹, R.W. Schafer¹, A.H.M. Burghes², T.W. Prior¹.* 1) Dept Pathology, Ohio State Univ, Columbus, OH; 2) Dept of Medical Biochemistry, Ohio State Univ, Columbus, OH.

Approximately 95% of spinal muscular atrophy (SMA) patients lack the SMN gene. The remaining 5% are assumed to be compound heterozygotes (deletion of one allele and mutation of the other). Point mutation analysis is usually reserved for SMA patients who are known to have one deleted allele. However, it is possible for a mutation in SMN to have a dominant negative effect, as shown by Pellizoni et al. (1998) who engineered deletion mutations in cell culture. We, therefore, screened 201 SMA-like deletion-negative patients for dominant-negative types of mutations by heteroduplex analysis. We did not identify any mutations in this population of patients indicating that dominant negative mutations do not play a significant role in causing an SMA-like phenotype. It is likely that other genes are involved in the pathogenesis of the remaining cases. Using a competitive dosage assay, we have investigated the role of new mutation events in SMA. 82 parents of simplex cases were tested for carrier status and 4 were shown to have normal two-copy dosage. The possibility of two tel-SMN genes in cis on the same chromosome is a confounding factor in carrier testing, because a parent can have apparently normal dosage yet transmit a null allele to their child. We are using a dosage assay on a human-mouse hybrid system to try to provide conclusive evidence for the existence of this genetic event. Lastly, an allele-specific oligonucleotide (ASO) based point mutation panel is being developed to increase the sensitivity of the SMA diagnostic test. There have been a limited number of mutations described in the SMN gene, so this screen would allow us to quickly detect compound heterozygotes. This work should have important implications for both the counseling and pathogenesis of SMA, as it would allow the routine diagnostic testing of compound heterozygotic patients and provide conclusive evidence for the existence of 2-tel/0-tel carriers as a distinct etiology from de novo deletion events.

Effects of fixation on DNA fragmentation and sequence alterations. *Q. Liang*¹, *W. Chu*², *B.H. Thompson*¹, *J.T. Simpson*¹. 1) Center for Medical and Molecular Genetics, Armed Forces Institute of Path, Rockville, MD; 2) Department of Hematopathology, Armed Forces Institute of pathology, Washington, DC.

Formalin fixation and paraffin embedding of tissue samples provide a convenient method for long term storage of fresh tissues. Analysis of nucleic acids from paraffin-embedded tissue blocks is crucial for clinical research and genetic studies of diseases. Though formalin is a traditional tissue fixation method, the time required for the fixation and the chemical modification involved in the process may lead to cross-linking and breakage in molecules such as protein, DNA, and RNA. A high frequency of sequence alterations has also been observed due to formalin fixation in archived specimens. A novel ultrasound-mediated high-speed tissue fixation method has been developed which provides excellent preservation of protein and RNA as well as morphology. Here, we compare this new method to conventional formalin fixation to evaluate the effects of fixation on DNA breakage and sequence alteration. Tissue samples will be prepared by routine formalin method and the new technique. DNA will be extracted from the paraffin blocks, amplified with primers bracketing various lengths of DNA fragments, and analyzed by direct DNA sequencing. The quantity and sequences of DNA fragments extracted from the samples will be compared.

Improvement of the method for molecular diagnosis of Machado-Joseph Disease. *M.C. Costa¹, C. Santos¹, A. Ferro¹, J. Sequeiros^{1,2}, P. Maciel^{1,3}.* 1) UnIGENe, IBMC, Univ do Porto, Portugal; 2) ICBAS; 3) ISCSN, Portugal.

Machado-Joseph disease (MJD) is caused by the expansion of a (CAG)_n tract, localized on the *MJD1* gene (7-47 repeats in normal genes, 56-86 repeats in mutated genes). The availability of a molecular method for direct mutation detection allows diagnostic and predictive testing of MJD. One of the difficulties of the direct test is the occurrence of *homoallelism*, i.e, homozygosity for two normal alleles with the same (CAG)_n length. The frequency of *homoallelism* in the Portuguese population is 20%, suggesting that in one out of five normal individuals it will be impossible to completely exclude non-amplification of an expanded allele, either due to an extremely large (CAG)_n or to the presence of polymorphisms in the primer-annealing regions of the gene (which may lead to false-negative results). To overcome this problem, we developed two alternative strategies: distinction of the two normal alleles using intragenic polymorphisms and a Southern blot based method. Three SNPs (GGG⁹⁸⁷/GGC⁹⁸⁷, TAA¹¹¹⁸/TAC¹¹¹⁸ and C¹¹⁷⁸/A¹¹⁷⁸) were analyzed, using allele-specific PCR, in 174 *homoallelic* individuals found in a large control population and in 21 *homoallelic* individuals from our diagnostic and predictive testing practice. This allowed for the distinction of the two alleles of normal size, but with different variants at these positions in 1% of cases for GGG⁹⁸⁷/GGC⁹⁸⁷, 54% for TAA¹¹¹⁸/TAC¹¹¹⁸ and 6% for C¹¹⁷⁸/A¹¹⁷⁸ in the control population. In our set found in routine testing of *homoallelics*, the distinction was possible in 10% of the cases using GGG⁹⁸⁷/GGC⁹⁸⁷, 86% using TAA¹¹¹⁸/TAC¹¹¹⁸ and 19% using C¹¹⁷⁸/A¹¹⁷⁸. We propose that a combination of the polymorphisms TAA¹¹¹⁸/TAC¹¹¹⁸ and C¹¹⁷⁸/A¹¹⁷⁸ should be routinely used in the diagnostic procedure of the MJD, to decrease the uncertainty created by *homoallelism*. Alternatively, a Southern blot method can be used digesting genomic DNA with the restriction enzyme AluI. This results in a single band from ~600 bp for normal alleles and in bands >733 bp for expanded alleles. A PCR-derived probe labelled with ³²P will hybridize with the restriction fragments upstream of the CAG repeat.

First year experience in genetic follow-up after implementation of cystic fibrosis newborn screening in

Massachusetts. *P.G. Wheeler¹, R. Smith¹, H. Dorkin², R. Parad³, A.M. Comeau³, D.W. Bianchi¹.* 1) Dept Pediatrics, Div Genetics, Tufts Univ Sch Medicine, Boston, MA; 2) Cystic Fibrosis Center, Div Pulmonology and Allergy, Dept Pediatrics, Tufts Univ Sch Medicine, Boston, MA; 3) New England Newborn Screening Program, Univ Massachusetts Medical School, Worcester, MA.

In February of 1999, Massachusetts began an expanded newborn screening protocol that included cystic fibrosis (CF) screening. The statewide screening program utilizes a two-tiered screening algorithm (IRT/DNA) modeled after the Wisconsin program and modified to include 16 CFTR mutations. Those infants who had a single CFTR mutation detected had a sweat test done, and their parents were offered genetic counseling. In the first year of the protocol, we saw 73 infants. Two infants (twins) had sweat tests consistent with CF. We counseled 69 families (3 families were previously counseled). Of the 69 couples offered CF carrier testing, 6 couples refused testing. In 9 couples, only 1 parent (the mother) was tested. Four of these women were carriers, and testing was recommended for the infant's father. In the remaining 54 couples, both parents were tested. In 49 couples, only 1 parent was a CF carrier; information on carrier risk in siblings and extended family members was provided. In 5 couples, both parents were CF carriers. They received counseling regarding their risk to have a child with CF. When both parents were carriers, CF testing was offered to their other children, and 1 sibling of a carrier infant was unexpectedly found to have CF.

The advantages of performing sweat tests in these infants and offering genetic counseling to their parents include: identifying a small number of infants with CF not detected by the 16 mutation analysis, finding couples at risk to have an affected child, identifying a previously undiagnosed sibling with CF, and potential identification of CF carriers in the extended family. The disadvantages of this testing include identification of a large number of infants (71 in our study) who are only CF carriers with at least 1 parent an obligatory carrier whether or not these individuals wish (or will wish) to know their carrier status.

Types of Referrals to Genetics Clinic from an ENT Clinic Following GJB2 Testing. *M.I. Roche¹, E.M. Rohlf², J.K. Booker², M. Zariwala³, L.S. Silverman², C.G. Shores⁴, C.M. Powell¹.* 1) Dept Pediatrics, CB #7220, Univ North Carolina, Chapel Hill, NC; 2) Department of Pathology and Laboratory Medicine; 3) Cystic Fibrosis Center; 4) Division of Otolaryngology.

Beginning in July 1999, direct testing for the GJB2 gene mutation, 35delG, has been offered to patients referred to the UNC Division of Otolaryngology for evaluation of hearing loss and possible cochlear implantation. Approximately 50% of recessive, nonsyndromic hearing loss is caused by mutations within this gene. The spectrum of referrals to the UNC Genetics and Metabolism Clinic resulting from this testing is reported. Sixty-six patients have had 35delG analysis followed by gene sequencing. Eight of these have been seen to date in genetics clinic and include 1 of seven 35delG homozygotes and 1 W24X homozygote. Both have prelingual, sensorineural hearing loss and have received successful cochlear implants. One of 8 heterozygotes has been seen and has prelingual, nonsyndromic hearing loss. Clinical genetics evaluation can be useful in clarifying results of heterozygotes but barriers include the recent availability of testing and the large catchment area.

A total of 12 patients, 8 without prior GJB2 testing, 4 of whom had testing after their genetics visit, have been seen and include a wide range of genetic and environmental causes of hearing loss. Three families (four patients) had a positive family history. Causes of hearing loss include Pendred syndrome, congenital toxoplasmosis, and possible Usher syndrome. One patient had hearing loss following a bone marrow transplant. Ototoxic drugs are suspected and analysis of the 12S rRNA mitochondrial gene mutation, A1555G, using skin fibroblasts, is planned. Some causes remain unidentified. One infant, suspected of having hearing loss, had an equivocal hearing test. After counseling, the parents declined GJB2 testing because of the uncertainty of the diagnosis and concern about possible insurance discrimination. Since fewer than 20% of referrals represented detected homozygotes, genetics clinics should prepare for a wide range of referrals following implementation of GJB2 testing.

Informed Consent for Human Genetics Research. *L. McCabe¹, W.W. Grody^{1,2}, B. Henker³, C. Jaenicke⁴, S. Peckman⁴, R.S. Sparkes⁵, F. Wiley¹, E.R.B. McCabe¹.* 1) Dept Pediatrics; 2) Dept Pathology; 3) Dept Psychology; 4) Office for Protection of Research Subjects; 5) Dept Medicine, UCLA, Los Angeles, CA.

Our goal was to provide guidance for investigators in using human samples. Genetic investigation involves all areas of clinical research, since genetic information is contained in sources as diverse as family histories and pathological specimens. Informed consent for human genetic research involves complex issues for research subjects and investigators. Issues include privacy and confidentiality of genetic information, sharing tissue and/or information with other investigators, feedback to research participants, and sample ownership. A Subcommittee of the UCLA Human Research Policy Board was charged with developing a consistent and complete approach to prospective human tissue research. The Subcommittee met to discuss published reports and guidelines from professional organizations on genetic testing, and to develop an appropriate informed consent policy to cover genetic research on human material. The Subcommittee included standard language in the Consent Form to explain information contained in DNA, RNA and protein. No relative would be contacted without the research subject's permission. If tissue remains at the end of the study, subjects can specify on a checklist if they will permit this material to be shared with other researchers or not. Participants can indicate their preference to receive information about their sample, general information about study results, or no information. In signing the Consent Form, subjects acknowledge that the University of California, or its designee, owns their sample. The Subcommittee prepared standard language for Informed Consent Forms resulting in a more informative and consistent consent process. The work of the UCLA Human Subjects Institutional Reviews Boards has been enhanced by this unified approach to genetic research. Investigators who are not geneticists have been sensitized to the issues of genetic testing of tissue samples. While the Subcommittee recognized that issues of genetic testing are evolving, the standard language was an attempt to deal with current issues and to anticipate future concerns.

A comprehensive genetic laboratory database: a critical tool[for community-based and statewide public health planning]. *J.C. Sullivan¹, T. Marini¹, R. Greenwood¹, R. Blatt², R. Naeem¹.* 1) Laboratory Genetics, Baystate Medical Ctr, Springfield, MA; 2) Department of Public Health, Boston, MA.

The need for comprehensive genetics laboratory data collection and information systems continues to grow alongside advancing genetic technologies. Aggregate genetics laboratory data at the community and state level can assist in determining rates of disease, utilization patterns, referral trends, gaps and inequities in access to services, and health outcomes. We are a genetics laboratory service based in a teaching hospital with a specimen volume of over 3000 cases annually. In 1996, we were confronted by the challenge of implementing a more efficient information system for electronically tracking our growing number of cases and organizing the data associated with each case. At the time, commercially available genetic databases were either too rudimentary to suit our needs or the costs associated with their implementation were prohibitive. Thus, we created our own database using Microsoft Access 2.0. Our goal in setting up this database was threefold. First, to monitor the flow of cases through the laboratory on a day to day basis. Second, to monitor specimen trends in order to efficiently manage and project laboratory workload and schedule personnel. Third, to establish and monitor quality assurance indices such as specimen turnaround time. This database is an important tool for monitoring test utilization patterns of our genetics referral base. It has helped us to continuously meet the genetic testing needs of our community by providing critical feedback and projections for future demands. As part of the Massachusetts public health genetics planning process, Baystate Medical Center, in collaboration with the Massachusetts Genetics Program/Department of Public Health, is developing a pilot project to identify key genetics data elements and develop the data tools critical for public health planning. The identification of these data elements, and the ability to track and monitor trends, will improve public health planning at the state and local level and will serve as a model for other states developing public health genetics capacity.

A model program to ensure genetic services are available for Washington State residents enrolled in managed care plans. *D.L. Doyle*. Genetic Services Section, Washington State Dept Health, Seattle, WA.

The *Partners' Forum* was initiated in Washington State in October 1997 to explore issues related to genetic services within managed care plans. The goals of this project were to: (1) establish a forum that would lead to improved communications among stakeholders interested in genetic services in managed care, (2) enhance access to quality genetic services, (3) improve linkages between primary care providers and genetic services providers, (4) make recommendations to address the varied and complex issues, (5) demonstrate cost-effectiveness and patient satisfaction with genetic services, and (6) develop tools and materials necessary to implement the recommendations generated. To meet these goals, stakeholders with varied interests and perspectives were convened. This model "think-tank" consisted of geneticists, primary care providers, consumers, case managers and medical directors from managed care plans, health care purchasers, Medicaid staff, the Deputy Insurance Commissioner, and public health representatives. These consultants were queried regarding their personal views on genetic services in a managed care environment and were tasked with identifying and prioritizing the issues. Six focus areas were described: (a) Availability of genetic services, (b) Quality, (c) Awareness of genetic services, (d) Accessibility (If services are available, are there barriers to accessing them?), (e) Affordability, and (f) Confidentiality. Eight forums were held with the Partners between January 1998 and June 2000. In addition, "in-between forum" activities were undertaken including convening a group of managed care case managers to solicit their perceptions of need for genetic services, data collection concerning actual reimbursement levels to genetics clinics, and a review of existing state regulations pertaining to consumer protections. The results of this model approach and its impact have included an increased request for genetics education for multiple target audiences, the initiation of a process for credentialing genetic counselors in plans, and an improved understanding between geneticists and case managers regarding referral and authorization issues.

Surfing the web for genetic information: Patient utilization of the Internet as an informational source in a general genetics setting. *M.R.G. Taylor¹, A. Alman², D.K. Manchester¹*. 1) Division of Genetics, University of Colorado, Denver, CO; 2) Department of Preventive Medicine and Biometrics.

The Internet has greatly expanded patient access to genetic information, but the impact of unfiltered data on clinical genetic practice is not yet understood. Our survey of Internet usage among families indicates that patients with genetic conditions commonly access the World-Wide-Web, searching for such information; and that they seek further direction for these efforts from clinicians. We developed a survey to assess Internet usage among our patients visiting urban and rural clinics in Colorado and Wyoming. We distributed 145 surveys, of which 115 (79%) were returned (49% rural; 51% urban). 54% of the visits were new patients. 46% were return visits. Respondents were over the age of 17; in 90% of cases this person was the patient's parent or guardian. Fully 44% of our patients had searched the Internet for genetic information prior to their clinic visit. The majority of such efforts were prompted by the patients (parents/guardians) themselves; only 8% had been referred to a web site by a physician. Interestingly, 93% of the respondents indicated that they would be likely to visit a web site that was recommended by a Geneticist. The most compelling reason to search the Internet was: to get information in laypersons terms (58%); to get information about treatment (13%); to get information about genetic research (13%). Among individuals who had actually visited Genetics-related web sites: 36% agreed that information was confusing or difficult to understand; 65% agreed that information was accurate and could be trusted; 80% agreed that using the Internet was a positive experience. These data highlight that Internet use is widespread and that a potential for misinformation exists. Patients commonly found some of the information confusing and questioned its accuracy. The majority of our patients said they would visit a web site recommended by a geneticist. However, referral to web sites by physicians seems rare. Further studies are needed to better establish the accuracy of Internet information and how to best integrate and/or accommodate this data into the Genetics clinic.

Partners in Research. *S.F. Terry^{1,2}, V.H. Whittemore^{1,4}, P.F. Terry², J.D. Cody^{1,3}, M.E. Davidson¹*. 1) Genetic Alliance, Washington, DC; 2) PXE International, Inc, Sharon, MA; 3) Chromosome 18 Registry & Research Society, San Antonio, TX; 4) Center Without Walls, National Tuberous Sclerosis Association, Landover, MD.

Based on decades of partnering with lay advocacy groups involved in research, the Genetic Alliance defines research as a shared enterprise - collaboration between participants and researchers. At the same time, the cultures of scientific investigators and lay advocacy groups differ, resulting in dynamic and creative tensions between shared and divergent interests. Lay advocacy groups are empowered to assume their role as equal partners in research. These groups represent research participants' interests - both those directly involved in the study and those who stand to be impacted by study results. Lay advocacy groups can enhance research progress by ensuring that research has a clear focus that will prove most effective for their disease interest and their families. Lay advocacy groups also can enhance research progress by educating the participants, the media, funding agencies and policy makers about the importance of the research. Lay advocacy groups are optimally positioned to directly collect epidemiological and phenotypic data. Because the lay advocacy group IS the community for most of the affected individuals with a specific genetic disease, they can access essential information that is usually inaccessible to clinicians or researchers who employ more traditional participant and tissue solicitation methods. Three models to be presented include: 1) research coordinated by the advocacy group at various sites where the advocacy group serves as the liaison between the research participants and the research consortium; 2) research conducted with the advocacy group where the researchers are all located at one research site; and 3) research performed by the advocacy group together with the researcher(s). The presentation of these models will also emphasize the partnership between the advocacy group and the researcher(s) in obtaining funding, obtaining informed consent from the participants; obtaining IRB approval, as well as publication of the results.

Results of a 36-nation survey of geneticists' ethical views. *J.C. Fletcher*¹, *D.C. Wertz*². 1) University of Virginia, Keswick, VA; 2) University of Massachusetts Medical School, Shriver Division.

In order to promote cross-national ethical discussion, we surveyed all practicing medical geneticists and genetic counselors in the 36 nations that had 10 or more geneticists, including 14 in Western Europe, 4 in Eastern Europe, 8 in Latin America, Israel, South Africa, Australia, China, India, Japan, Thailand, Turkey, Canada, and the USA. Colleagues in each nation distributed and collected the anonymous questionnaires, which included 50 ethical questions, mostly in the form of case vignettes. Of 4594 asked to participate, 2901 (63%) responded. Major global findings fall under 11 headings: 1. Most geneticists would give purposely biased information after prenatal diagnosis, except in English-speaking nations (Australia, Canada, South Africa, UK, US) and parts of Western Europe. 2. Most geneticists take a pessimistic view of disability. 3. There is a growing tendency to respect patient autonomy. 4. In most of the 19 nations previously surveyed in 1984, more would perform prenatal diagnosis for sex selection or refer than in 1984. 5. As in 1984, there was no consensus about telling relatives at genetic risk against a patient's wishes. Except in English-speaking nations and western Europe, majorities would tell relatives. 6. Geneticists in English-speaking nations would not test children for adult-onset disorders. Elsewhere, most would test children at parents' request. 7. There was worldwide consensus that employers, insurers, and schools should not have access to genetic information without an individual's consent. 8. Eugenic thinking persists in many developing nations, especially in Asia and Eastern Europe, but most reject involuntary sterilization. 9. There is a growing tendency toward full disclosure of genetic information to patients. 10. Most geneticists would not disclose an accidental finding of nonpaternity to a husband, even if he asks. 11. There were few reports of refusals of employment or insurance outside the US. In the US, reports were few when compared with patient volume. Results indicate a need for international discussions that also include the views of users of genetic services. .

Collecting patients and families for gene disease identification : the cell and DNA core facilities in Marseille (sponsored by the Association Francaise contre les Myopathies). *A. Navarro, P. Negre, D. Taulier, V. Labelle, V. Ravix, J.F. Mattei, N. Levy.* Departement de Genetique Medicale, Banque de cellules et d'ADN, Hopital de la Timone, Marseille, France.

Cell and DNA banks have been used for collecting patients and families affected with genetics disorders. The aims of such structures were and still are to identify genes involved in genetics disorders. Here we present the cell and DNA bank created in 1995 in Marseille. This structure has now collected 2700 samples from patients and families. The largest collections concern neuromuscular disorders, male infertility, mental retardation, ophtalmologic disorders,... Many rare disorders have also been collected. The collections that have not been studied by research groups will now be available to the international research community. Our structure can provide DNA, lymphocytes or immortalized lymphoblasts after validation of a resarch project by the scientific comitee. A web site is under construction and will be presented. The pages will present all the collections disponibles and also the current projects; this should enhance collaborations between research groups. Finally, we strongly believe that the cell and DNA core facilities will now be used more efficiently since the sequencing of the Human genome has been achieved and will be released in public databases.

Pharmacogenetics: Consideration of Ethical, Legal and Social Implications. *E.C. McPherson¹, A.E. Buchanan², P.K. Manasco¹*. 1) Dept Clinical Genetics, Glaxo Wellcome, Res Tri Pk, NC; 2) Dept Philosophy, Univ Arizona, Tucson, Az.

Advances in pharmacogenetic research are changing the paradigm of how medicines will be developed, tested, marketed and in particular, prescribed. These changes will affect all stakeholders of the healthcare system. Little empirical information is available relating to the ethical, legal and social implications (ELSI) of applying pharmacogenetics to the provision of healthcare. Ethics policy groups at all levels have defined guidelines regarding the use of genetic testing to diagnose and predict disease including guidelines regarding privacy/confidentiality, informed consent, risk/benefit assessment, patient/family counseling and potential for discrimination and stigmatization, but have not provided guidance specific to pharmacogenetics. In an effort to carry out meaningful dialogue with those who shape public policy, Glaxo Wellcome scientists collaborated with external ethics policy consultants to identify the potential ELSI concerns associated with pharmacogenetic research. The type of information obtained from the different types of genetic tests and the potential ELSI implications of each were examined. The principal aim of pharmacogenetics is to understand the genetic basis of patient's responses to medicines so that treatments can be targeted to those most likely to benefit. Thus, the information produced from a pharmacogenetic medicine-specific profile differs from the information produced from 'gene-specific tests' designed to diagnose or predict a risk of disease in non-symptomatic individuals. Based on this exploratory examination, the magnitude of ELSI concerns is less for predicting individual response to medicine than for disease gene-specific tests. The ELSI concerns associated with genetic testing to diagnose and predict disease are often driven by the lack of effective interventions. Potential pharmacogenetic ELSI concerns include: lack of provider and patient knowledge, oversight of marketing practices, accuracy and interpretation of profiling and the possibility for discrimination due to collateral information and increased healthcare cost for non-responders. These issues require further discussion by ethics policy groups.

Comparing Knowledge and Attitudes of Primary Care Physicians, Residents and Medical Students Regarding Genetic Testing and Related Ethical Issues. *A.E Roberts, M.J. O'Neill, M.A. Clay, L.A. Demmer.* U. Mass. Memorial Health Care, Worcester, MA.

Most primary care providers are not comfortable with their current knowledge of expanding genetic services, nor with the ethical questions raised by this new technology. This is the first study to compare resident and student knowledge of genetic testing with that of practicing physicians.

A survey was mailed to 161 primary care residents and 417 students from the UMass Med School and 1000 primary care MDs in Mass. There was a 58% response rate after two mailings. Questions included demographics; sources of education in genetic testing and ethics; referral patterns, and a series of ethics questions regarding genetic testing.

The majority of respondents reported feeling uncomfortable with their knowledge of available genetic testing(64%), did not feel they had a standard for deciding when patients need to be informed about the option of genetic testing(71%), and were not comfortable counseling patients before and after genetic testing(62%). Older respondents and male respondents were more likely to feel comfortable in their knowledge and abilities, however these same groups scored lowest on the knowledge-based questions. Contrary to most geneticists, the majority of respondents would authorize carrier testing of minors for cystic fibrosis(51%), and 35% would authorize testing of minors for Huntington Disease. Students and residents had the highest scores on the ethics questions. They were not more likely to feel comfortable with their abilities regarding counseling. 81% of respondents would want to participate in an educational program on genetics and ethics.

CONCLUSIONS:Primary care providers desire more training regarding genetic testing and ethics. Medical students and residents demonstrated superior knowledge compared to practicing physicians but still report feeling ill equipped to deal with this complex issue. Educational efforts regarding genetic testing should continue to expand at both the undergraduate and graduate level.

Identification of barriers to the provision of genetic services to an under served urban population. *H.M. Saal, L. Hoechstetter, C. Hetteberg, E.K. Schorry.* Division of Human Genetics, Children's Hospital Medical Center, Cincinnati, OH.

In 1998 monthly genetic clinics at three community health centers located in poor urban areas in Cincinnati were established. These clinics were initiated because it was recognized that African Americans were grossly under represented in the genetics outpatient population in our institution. As an adjunct to the clinics, an educational curriculum for the health professionals at the clinics was developed in order to improve genetics knowledge and increase the number of referrals for genetics services. Review of the data from patient visits for the first 16 months of the program shows a significant number of African American patients seen at the urban genetics clinics. We compared racial characteristics of patients seen at the urban genetics clinics to those of genetics outpatients at Children's Hospital Medical Center (CHMC). There were 914 patient visits by 747 patients at CHMC, of whom 636 patients were Caucasian (85.1%) and only 79 were African American (9.4%). These data were consistent with those from prior years. In contrast, at the three urban genetics clinics, there were 86 visits by 65 patients, of whom 15 were Caucasian (23.1%) and 45 were African American (69.2%). Most patients evaluated at CHMC (94%) and at the urban genetics clinics (98%) had health care coverage, excluding this as a barrier to genetics services. The three community health centers represent only a small portion of the community health care clinics in metropolitan Cincinnati. These data support the need for developing a network of genetics services in neighborhood clinics in order to reach under served populations. Further analysis is under way to determine the roles of health care professional education, transportation assistance, and nursing case management services in improving clinical genetics services for these populations. Supported by Maternal and Child Health Bureau grant MCHG 98-07.

Economics of clinical genetics services: Impact of a connective tissue disorder clinic. *D.L. Thull¹, M.K. Bourdius¹, S.L. Sell¹, R.E. Pyeritz^{1,2}*. 1) Allegheny General Hosp, Pittsburgh, PA; 2) MCP Hahnemann School of Medicine.

In 1992, we documented that the economic impact of a range of cognitive genetic services was much greater to the institution as a whole than to the clinical geneticists. Many changes continue to shape the use of consultation, including managed care, the need for authorizations for services, restrictions on providers of testing and procedures, and levels of reimbursement for both cognitive and procedural services. To determine if any systematic trends have emerged in recent years, for 1995, 1997 and 1999 we examined: the charges attributable to genetics consultation and to other medical services rendered by the institution as a direct result of the genetics consultation; reimbursement of these charges; and, limitations placed by insurers on both the type and provider of other services. The site was an outpatient clinic that specialized in heritable disorders of connective tissue in a tertiary-care, urban, academic hospital. Data on 304 patients were collected. Charges for cognitive genetics services were \$228.50, 264.40 and 210.00, with the differences due to variations in the level of service/complexity and a modest increase in charges/level of service. Reimbursement data were extremely difficult to obtain retrospectively. Most patients required echocardiography; the fraction who were not permitted by insurance to have this test at our institution was 31, 35 & 35% in the 3 years. Additional services (specialist consultations, radiology, surgery) provided by our institution resulted in charges of \$714, 1632 & 1710 per patient in these 3 years. Additional institutional charges relative to genetics charges increased from 3.1 to 6.2 to 8.1-fold, numbers that would have been considerably greater had not some patients had MR imaging and surgery elsewhere. We conclude that outpatients referred to a clinical geneticist for cognitive services related to connective tissue disorders continue to generate substantial revenue in excess of fees for genetics services, and that as a consultative service matures, this factor grows. In order to generate reliable data on reimbursement in our institution, prospective collection of data would be necessary.

Characterization of microsatellite markers flanking *FBNI*: utility in the diagnostic evaluation for Marfan

syndrome. *D.P. Judge*¹, *N.J. Biery*², *H.C. Dietz*^{1,2,3}. 1) Department of Medicine, Johns Hopkins University, Baltimore, MD; 2) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 3) Howard Hughes Medical Institute.

Marfan syndrome (MFS) is an autosomal dominant disorder of connective tissue characterized by marked interfamilial and intrafamilial variation in phenotype. The primary defect in affected patients resides in the gene for fibrillin-1 on 15q21 (*FBNI*). Substantial allelic heterogeneity and the large size of the gene (200 kb) preclude routine mutation screening in presymptomatic, prenatal, and equivocal cases. We have previously shown that four intragenic microsatellite markers may be used for haplotype segregation analysis. This approach is limited by incomplete spanning of the appropriate region and incomplete informity for the described markers, with 16% homozygosity for the most common haplotype. We now demonstrate the relative position of 4 flanking microsatellite markers extending from 800 kb 5' to 300 kb 3' to the coding region. These markers show complete haplotype heterozygosity in a population of fifty unrelated unaffected people. We demonstrate the utility of these markers for presymptomatic and prenatal diagnoses when the intragenic markers are uninformative, and confirm the validity of the diagnostic criteria for MFS in family members with suggestive, but not diagnostic manifestations. These markers were used for the analysis of a family that provided evidence for locus heterogeneity in classic MFS. Extended haplotype segregation analysis confirmed that an individual with subtle eccentric placement of the ocular lens and an aortic root measurement above the 95th percentile did not inherit the *FBNI* allele shared by 6 relatives with classic MFS. Linkage analysis was entirely consistent with the involvement of *FBNI* in the extended family. Mutation analysis confirmed that the proband did not carry the genetic predisposition for MFS present in the extended family. These data caution against overinterpretation of clinical findings that can overlap with the normal population, a tendency with a family history of MFS.

MTHFR genotypes, NTD risks, and Downs risks during pregnancy. *K. Yanamandra, D. Napper, T.F. Thurmon, H. Chen, S. Ursin, J.A. Bocchini, Jr.* Dept Pediatrics, LSU Medical Ctr, Shreveport, LA.

Role of the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) has been implicated in the etiology of cardiovascular disease, neural tube defects (NTD) and Down syndrome. However, reports on the relationship of MTHFR genotypes and poor pregnancy outcome through mid-trimester triple screening (MSAFP, MSHCG and MSuE3) were lacking. Here we studied local pregnant women, who went through our triple screening program for NTD and chromosomal defects, for the presence or absence of MTHFR mutation C677T. Out of more than 1000 pregnant women in their second trimester that were screened for the mutation, we found that Hispanic pregnant women had the highest mutant homozygosity followed by the Caucasians and least by African-American women. Higher heterozygosity and homozygosity with ethnic differences for the mutation were observed among women who were at high risk for NTD and Down syndrome pregnancy outcomes. Data and statistics with the breakdown among various ethnic pregnant population will be presented.

Use of a Stool Based Screening Assay for High-risk Individuals. *S.J Laken¹, D.A Ahlquist², J. Jen³, K. Boynton¹, A.P. Shuber¹.* 1) Exact Laboratories, Maynard, MA; 2) Mayo Clinic, Rochester, MN; 3) Johns Hopkins Medical Institution, Baltimore, MD.

Patients with HNPCC are at an increased risk for developing colorectal cancer (CRC) and it is recommended that colonoscopic surveillance be performed every one to three years. Though most CRC will be detected early by colonoscopy, some individuals will still develop cancer in the intervening years between their examinations. A non-invasive, stool based colorectal cancer screening test is currently being developed by EXACT Laboratories. One component of this assay examines stool DNA for BAT-26 deletions within intron 5 of hMSH2. In three blinded clinical studies, BAT-26 mutations were found in 12 of 100 stool specimens from known cancer patients, while 0 of 105 colonoscopy negative individuals were negative. As this assay is accurate and specific, there may be applications in fecal DNA screening of high-risk HNPCC patients not being screened by colonoscopy, or for intervening screening between scheduled colonoscopic examinations.

Detection of Aerodigestive Cancers by Analyzing the Integrity of Human DNA Purified from Stool. *A.P. Shuber¹, D.A. Ahlquist²*. 1) Exact Laboratories, Inc, Maynard, MA; 2) Mayo Clinic, Rochester, MN.

Aerodigestive cancers account for > 50% of all malignant deaths. An assay for mutated DNA exfoliated into stool represents an intriguing approach to the noninvasive detection of such cancers. The development of a DNA integrity assay (DIA) that targets high molecular weight DNA appears to be a discriminant fecal marker for the detection of colorectal neoplasia (Gastroenterology, Ahlquist et al. in press 2000). However, such an approach to screening remains unexplored for more proximal cancers. To assess the potential of DNA integrity as a fecal marker for aerodigestive cancers, freezer-archived stool was assayed in blinded fashion from patients diagnosed with lung, pancreatic, and colorectal cancer, as well as colonoscopically-normal controls without history of neoplasia. The DNA Integrity Assay detected 6 of 8 (75%) lung cancers, 14 of 27 colon cancers (52%), and 5 of 7 (71%) pancreatic cancers, while retaining a specificity of 97% (75 of 77) within the normal control population. These observations represent proof-of-principle that aerodigestive cancers may be detected by utilizing DNA integrity as a general stool marker, and larger clinical studies are indicated to further explore this noninvasive screening approach.

Quantification of homologous alleles and methylation analysis by single-nucleotide primer extension and fluorescence-based DHPLC. *H. Yoshihashi¹, R. Kosaki^{1,2}, T. Suzuki¹, Y. Ohashi³, N. Matsuo¹, K. Kosaki¹.* 1) Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan; 2) Health Center, Keio University, Tokyo, Japan; 3) Transgenomic Japan, Inc, Tokyo, Japan.

The single-nucleotide primer extension [SNUPE] is widely used for the analysis of the differential expression of homologous alleles involved in genomic imprinting, X-chromosome inactivation, and allelic imbalances associated with cancer. In the SNUPE reaction, a primer that binds adjacent to the nucleotide polymorphism is extended by a single base in the presence of a dNTP and/or ddNTP specific for one of the variant alleles. The purpose of the present study was to evaluate whether quantitative SNUPE assay could be accomplished by fluorescence-based DHPLC. ROX-labeled dideoxy CTP (ROX-ddCTP) was incorporated at the 3' end of the primer annealed to the template in the SNUPE reaction. Then the product was loaded onto a DNasep column with a linear gradient of acetonitrile in triethylammonium acetate. Two separate peaks, one from the primer extension product, and the other from the unincorporated dye terminator, were detected. When mixed template with variable amounts of the T and C alleles was analyzed, the signal intensity from incorporated ROX-ddCTP correlated with the relative molar proportion of the C-allele in the genomic DNA template. Furthermore, fluorescent SNUPE reaction has been successfully utilized to assess degree of cytosine methylation. Genomic DNA was subjected to bisulfite treatment, by which unmethylated cytosines are converted to uracils whereas methylated cytosines are not. Using the principle outlined above, the relative molar ratio of the methylated alleles versus the unmethylated allele was accurately determined. Unlike the original SNUPE assay based on radio-labeling, chromatographic separation can be monitored on-line, and completed in less than ten minutes after automated loading of the samples. The development of high-throughput technology for quantitative analysis of allele-specific expression and methylation should significantly expand our ability to derive molecular information from clinical specimens.

Rapid Detection of Rare Mutations in Multiple Genetic Disorders Using DHPLC. *Z. Zhou, R.G. Pace, K.J. Friedman, M. Zariwala, P.G. Noone, M.R. Knowles, L.M. Silverman.* Dept Pathology & Lab Medicine and Dept Medicine, Univ North Carolina, Chapel Hill, NC.

Denaturing HPLC (DHPLC) is a highly sensitive method for detecting sequence variants in heterozygotes. Previous studies have shown detection rates ranging from 90% to 100%. We tested Varian's ProStar Helix DHPLC system for sensitivity and specificity. Each of the 39 known variants (37 point mutations and 2 small deletions) evaluated possessed a distinct absorption pattern compared with negative controls and other variants nearby. The sensitivity was 100% when more than one melting temperature was used for some samples. There were no false positives. To facilitate clinical diagnoses and carrier identification, we applied DHPLC to screening for rare CFTR mutations in patients with cystic fibrosis (CF). To date, there are 915 reported mutations scattered throughout the gene (27 exons). Patients with only one or no mutation are common. Full gene sequencing is both time-consuming and costly. Screening of samples with DHPLC followed by sequencing of the DNA fragments with altered DHPLC patterns is an effective and economical way to identify new mutations. With this strategy, we identified numerous rare mutations rapidly at low cost in CF patients with only one mutation identified by routine clinical tests of 6-70 mutations. We also used DHPLC to screen carriers for known mutations or variants in other genes, e.g., 3 variants (Z, S, and M3) in the α 1-antitrypsin gene, mitochondrial mutations associated with MELAS and MERRF, and mutations in the medium chain acyl-CoA dehydrogenase (ACADM) gene. Each variant produced a distinct and highly reproducible DHPLC pattern. More recently, we have discovered 2 novel mutations (W468S and W468X) in the dynein IC78 gene in patients with primary ciliary dyskinesia (PCD). After screening 125 normal controls for these mutations, we identified one W468X carrier by DHPLC with confirmation by sequencing. We conclude that rapid sample processing, low cost, and automated operation make DHPLC an ideal tool of detecting heterozygosity for rare mutations in patients and for screening carriers for known mutations, especially in a large patient volume.

Determination of the factor V Leiden (G1691A) and prothrombin (G20210A) mutations by using a dHPLC

system. *W.L. van Heerde¹, H. Kenis¹, P. Lux¹, K. Hamulyak³, D. Wi³, J.M.L.S. Lavergne², C.P.M. Reutelingsperger¹.*
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During the last five years two rather common mutations were discovered with a high prevalence of venous thrombo-embolic events. These mutations, the factor V Leiden (G1691A) and the G20210A prothrombin mutation are nowadays routinely screened in all coagulation laboratories. Several approaches have been used to determine these mutations, such as restriction enzyme analysis, Taqman and FRET technologies. Here we present an alternative methodology to determine both mutations in one single assay, by using the dHPLC system. In this system mutations can be determined on basis of the melting behaviour of heteroduplexes which elute from the column by a combination of column temperature and acetonitrile gradient. 155 subjects were included both symptomatic patients with proven venous thrombo-embolism and asymptomatic first degree relatives. Genomic DNA was amplified in a multiplex reaction and assayed on the system. To determine homozygosity all samples were also mixed with a negative control in a second run. A 100 % sensitivity was obtained for both mutations when compared to routinely screened assays. Moreover, we observed additional polymorphisms; 4 for factor V exon 10 and 2 for prothrombin 3 untranslated region. In conclusion, this alternative methodology is a reliable technique to determine both mutations in a routinely setting. This technique is fast and cost effective and can be performed on a per patient basis. Furthermore, it allows high throughput screening. Due to the presence of possible other polymorphisms we suggest to perform sequencing on positive samples. Finally, this multiplex screening can be adapted to other genes involved in thrombo-embolism.

Crosslinking hybridization assay for hemochromatosis gene mutations in a German population. *R. Peoples¹, P. Cheng¹, B. Huan¹, R.B. VanAtta¹, M.L. Wood¹, C. Wylenzek², M. Engelmann², D. Holten², B. Gathof².* 1) NAXCOR, Menlo Park, CA; 2) University Hospital Of Cologne, Germany.

Hereditary hemochromatosis is an autosomal recessive disorder characterized by progressive multiorgan iron overload. Homozygosity for the C282Y mutation and, in some cases, compound heterozygosity for the C282Y/H63D mutations confer the disease phenotype. We have developed direct assays for the identification of the C282Y and H63D mutations. In contrast with PCR-based tests, our method does not require amplification of target DNA sequences.

Each assay utilizes two DNA probe sets that contain reporter probes complementary to the HFE gene and one of two allele-specific capture probes. All probes are modified with UV-activated crosslinking molecules. The two capture probes are complementary to the sequence surrounding the mutation site, but differ at the mutation codon with respect to mutant or wildtype, thus enabling discrimination between the normal and mutant genotypes. Lysed blood samples are analyzed in a microtiter plate format in less than five hours. We have applied this assay to an asymptomatic German population of 632 according to a protocol in which all samples are first assayed for the C282Y mutation; heterozygotes are then assayed for the H63D mutation on the alternate allele. The first 100 individuals were assayed in parallel with a standard DNA test, with complete concordance between both methods. To date, screening has been completed in 310 cases. Of these, 23 C282Y/wildtype heterozygotes and 6 C282Y/H63D compound heterozygotes have been identified. Of the remaining 322 individuals, 32 are heterozygous for the C282Y mutation; H63D results are pending. No C282Y homozygotes have been identified. The C282Y/H63D compound heterozygotes are referred for clinical evaluation, including transferrin saturation, serum ferritin and serum iron concentrations.

The crosslinking method is a fast, cost-effective and reliable alternative to PCR in population screening for the HFE C282Y and H63D mutations. Clinical correlation with compound heterozygosity will be forthcoming.

Denaturing High Performance Liquid Chromatography (DHPLC) used in the detection of mutations and polymorphisms in the UBE3A gene. *D. Bercovich, E. Lev-Lehman, A.L Beaudet.* Human and Molecular Genetics, Baylor college of medicine, Houston, TX.

One of the most sensitive method for detecting point mutation causing human disease is PCR followed by direct sequencing. However, sequencing of those genes is technically demanding, costly, and time consuming; in addition, heterozygous mutations may go undetected. There are reports that Denaturing High Performance Liquid Chromatography (DHPLC) is as sensitive as direct sequencing for detection of point mutations, and we have evaluated the ability of DHPLC to detect known mutation in the UBE3A locus, which encodes E6-AP ubiquitin-protein ligase. Large deletions, imprinting defects or loss-of-function mutation in the maternal allele for UBE3A cause Angelman syndrome (AS), which is characterized by mental retardation, absence of speech, seizures and motor dysfunction. We studied 17 mutations in the UBE3A gene that was identified by Fang et al (Hum. Mol. Genet. Vol 8, 129-135, 1999), two new mutations and two polymorphic sites. The UBE3A gene is a very AT rich locus so we compared the use of primers designed for sequencing analysis and not for the DHPLC to primers with GC clamps. In some of the fragments, the use of GC clamp was necessary to detect the DNA alteration (exon 13/14, 16), and in some fragments (exons 8,9, 15) the primers designed for sequencing were adequate. Because the fragments are AT rich, the gradient temperature was always above the T_m of the fragments (+ 1 to 5 degrees). DHPLC detected 100% of the DNA alterations, and 30 controls samples were scored correctly. In addition, DHPLC allowed us to discriminate between different alterations in a single fragment, because of the characteristic elution profiles of the DNA molecules. In some fragments, 10-bp GC clamp showed DNA alteration in controls, probably due to Taq polymerase mismatch in amplification, and the GC clamp was reduced to 5-bp. We conclude that DHPLC is a highly sensitive, efficient and economical method to screen for point mutation in Angelman syndrome.

Determination of the frequencies of the $\Delta F508$, G542X and W1282X mutations in Iranian Cystic Fibrosis patients. *E. Elahi*¹, *F. Ghasemi*¹, *A. Khodadad*², *M. Houshmand*³. 1) Dept of Biological Sciences, Tehran University, Tehran, Iran; 2) Pediatric Polyclinic, Children's Hospital Medical Center, Tehran University, Tehran, Iran; 3) National Research Center of Genetic Engineering and Biotechnology, Tehran, Iran.

The frequencies of three mutations, $\Delta F508$, G542X and W1282X, in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on 100 unrelated CF chromosomes of Iranian Cystic Fibrosis patients were determined using the Amplification Refractory Mutation System-PCR (ARMS-PCR) protocol. The frequencies of these mutated alleles were found to be 16%, 1% and 5%, respectively. The very low frequency of the $\Delta F508$ mutation compared to other countries is consistent with its previously reported decreasing gradient from northwest Europe toward the southeast. Its reported frequencies in Denmark, France, Italy, Algeria and Turkey are, respectively, 88%, 69%, 46%, 43% and 18.8%. The G542X mutation frequency found amongst Iranians (1%) seems to be somewhat lower than that of Mediterranean countries such as Greece (4%) and Italy (2.8%) and also of Turkey (4.9%). The frequency of the W1282X mutant allele on Iranian CF chromosomes (5%) was higher than that of many countries, including England (0.2%), Spain (0.78%), Greece (0.4%) and even neighboring Turkey (0.8%). The frequency of this mutation amongst the Ashkenazi Jews of Israel is 60%. As judged by its history, the Iranian population is expected to be genetically very heterogeneous. The presented data will be relevant in designing an appropriate molecular biology protocol for diagnosis of CF in Iranian patients.

A Human Genomic DNA Bank as a Renewable Source of Genotype-Specific DNA Samples. *M.J. Palmer¹, X. Hu², M. Frantz¹, P. Pingerelli¹, B. Belachew¹, L. Molinari³, J. Belmont³, J. Kurth², D. DuBois¹.* 1) Molecular Medicine, Stratagene, Cedar Creek, TX; 2) Molecular Medicine, Stratagene, La Jolla, CA; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Genetic polymorphisms are increasingly used as a tool to assess predisposition to disease, and to predict the efficacy of therapeutic regimens. Validated human genomic DNA samples with specific genotypes are important both in diagnostic testing and in the development of assays for specific genetic tests. Unfortunately, many genotypes of interest are rare in random populations and DNA sources are often not renewable.

We have established a bank of cryopreserved lymphocytes and purified DNA from over 1000 individuals that can be repeatedly tested for specific genetic polymorphisms using high throughput screening technology. DNA from each individual is screened in a single PCR reaction containing the DNA template, PCR reagents, and two allele-specific molecular beacon probes. One allele-specific molecular beacon is labeled with the fluorophore tetrachloro 6-carboxyfluorescein (TET) and the other allele-specific molecular beacon is labeled with 6-carboxyfluorescein (FAM). A single fluorescent signal indicates the presence of only one allele (homozygote), while detection of both fluorophores indicates the presence of both alleles (heterozygote).

To provide a continuous source of genomic DNA, lymphocytes from individuals with a variety of clinically relevant polymorphisms have been immortalized with Epstein-Barr Virus (EBV). Genomic DNA is purified from the resulting cell lines, and the genotype confirmed by dye terminator DNA sequence analysis. Thus far, we have developed renewable sources of genomic DNA for a number of genetic polymorphisms, including CCR2 (I-64), CCR5 (del32), SDF-1 (3'-A), CFTR (del508), HFE (C282Y), and CYP3D6 (G1934A). Allelic discrimination kits for these genetic polymorphisms are also available from Stratagene.

Molecular screening and prenatal diagnosis for Finnish congenital nephrosis in low risk pregnancies in Finland.

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Objectives. The main objective was to identify previously undiagnosed individuals with Finnish congenital nephrosis (CNF) mutations so that they could be properly informed and offered prenatal testing. The autosomal recessive, serious disorder is one of 30 Finnish heritage diseases. CNF has been found to be caused by mutations in the nephrin gene. The most common Finnish mutations covering 95% of all cases are Finmajor (deletion in exon 2) and Finminor (nonsense mutation in exon 26). **Study methods.** From January 1999 ultrasound-screening test for nuchal translucency was offered to pregnant women of Kuopio City at the Kuopio University Hospital in the first trimester. Practically all women participated in ultrasound screening. DNA testing by PCR for the two CNF mutations was offered free of charge and on a strictly voluntary basis to all women. 96% of eligible women accepted the gene test. All women found to be carriers underwent genetic counseling and screening of the partner were offered. If the both partners would appear to be carriers, prenatal testing was offered. **Results.** A total of 1,186 women elected to undertake the gene test for FCN. 38 carriers for CNF-gene mutations were found, representing a carrier frequency of 1:31. 34 women had the Finmajor mutations and 4 women Finminor mutation. One of the tested husbands was also a carrier and prenatal diagnosis revealed an affected fetus. **Conclusions.** Trisomy screening is moving to first trimester, which means the end of AFP screening for CNF in Finland. This is compensated with the gene test, which is more accurate than AFP screening, and needed only once in lifetime. The gene test policy provides an effective way of identifying carriers of genetic diseases and incorporating prenatal testing into this process. Those women registering for CVS or amniotic fluid sampling should at least consider carrier screening.

Novel mutations in genes for frequent X-linked metabolic diseases (X-ALD, Fabry disease, OTC deficiency). A prevalent mutation for late onset form of OTC deficiency. *L. Dvorakova¹, G. Storkanova¹, R. Dobrovolny¹, M. Hrebicek¹, S. Kmoch¹, J. Ledvinova¹, M. Elleder¹, J. Zeman¹, J. Bultas²*. 1) Institute for Inherited Metabolic Disorders, 1st Faculty of Medicine, Prague, Czech republic; 2) Second Department of Internal Medicine, General Faculty Hospital, Prague, Czech Republic.

We have set up methods for mutation analysis of common X-linked metabolic disorders - X-ALD, Fabry disease and OTC (ornithine carbamoyltransferase) deficiency. As the majority of mutations are private, we start the analysis by direct sequencing of PCR or RT/PCR products amplified from probands gDNA or cDNA. Then we use specific PCR-RFLP or ARMS assays for genotyping of family members, genetic counselling and prenatal diagnosis. X-ALD is characterized by impaired peroxisomal very long chain fatty acids β -oxidation. The associated gene encodes a peroxisomal membrane protein (ALDP). We have analysed 11 unrelated patients with X-ALD and found 8 novel mutations. Fabry disease, a disorder of glycosphingolipid catabolism, results from mutations in the α -galactosidase A gene. In a series of 11 probands we have found 9 novel mutations. OTC deficiency is the most common inborn disorder of the urea cycle. In 2 patients with neonatal form of OTC deficiency we have identified 2 novel nonsense mutations. In 4 unrelated families with late onset form of OTC deficiency, common R277W missense mutation occurred. This mutation was reported to cause the late onset of OTC deficiency in an American family. This mutation has not been detected on more than 110 control alleles investigated. Our result suggests that R277W may be the prevalent mutation associated with late onset form. We have screened 54 family members (25, 13 and 16 from X-ALD, Fabry disease and OTC deficiency families, respectively) for the family-specific mutations. We have identified 29 heterozygotes. On the contrary, heterozygosity was excluded in 14 females. One carrier having an OTC deficiency and 7 carriers having Fabry disease manifest clinical symptoms. We have detected two presymptomatic boys hemizygous for R277W mutation in OTC gene. This knowledge may save life of both boys in the future.

Assay-dependent differences in the population distributions of five unconjugated estriol methods, and their effect on prenatal screening performance. *C.M. Balion¹, A.R. MacRae²*. 1) Department of Pathology and Laboratory Medicine, Children's & Women's Health Centre of British Columbia, Vancouver, British Columbia, Canada; 2) The Research Institute at Lakeridge Health, Oshawa, Ontario, Canada.

Low concentrations of maternal serum unconjugated estriol are associated with an increased risk of Trisomy 21 and Trisomy 18. In prenatal screening programs that utilize estriol, the second trimester maternal serum unconjugated estriol (uE3) concentration is measured in combination with the levels of alpha-fetoprotein and chorionic gonadotropin. Combined patient-specific estimates of risk of the target disorders are calculated using various mathematical approaches, most commonly a likelihood ratio (affected:unaffected) from multiple of the unaffected median (MoM) Gaussian distributions. The analytical performance of each of the markers is a factor in the final screening performance. We have evaluated five uE3 methods on the basis of their imprecision and their Gaussian population distributions (mean and SD). Fresh and thawed mid-trimester sera from pregnant patients electing to participate in two established screening programs were analysed anonymously using five uE3 reagents from four sources: EG&G Wallac (W1-original; W2-reformulated), Bayer Diagnostics, Beckman Coulter, and Ortho Clinical Diagnostics (Amerlex-M). For three reagents the performance was also assessed in a fully outcome-ascertained Trisomy 21 population. In the unaffected population, Amerlex, Beckman Coulter and Bayer methods were not significantly different from each other in their population distributions (spread of data). In contrast, W1 and W2 were significantly different from these three methods and from each other. In the Trisomy 21 group there was agreement between the Amerlex and Beckman Coulter methods, but not with the Bayer method, and this difference was reflected in a diminished detection rate with the Bayer method. Our data show that conversion of absolute assay values to MoMs does not eliminate interassay variability, and the population distributions of the assays are not always consistent with risk calculation algorithms in use. This could cause unexpected changes in the calculated risks and screen positive rates.

Is there a problem with the current practice of using US dating in the prenatal screening programs for birth defects? *D. Napper, H. Chen, T.F. Thurmon, S. Ursin, J.A. Bocchini, Jr, K. Yanamandra.* Dept Pediatrics, Louisiana State Univ Med Ctr, Shreveport, LA.

LSU medical school in Shreveport, Louisiana began offering screening for NW Louisiana pregnant women since the beginning of 1995 for birth defects using triple screening method. MSAFP MoMs in the detection of NTDs were adjusted for maternal weight, race, and IDDM. However, MShCG and MSuE3 MoMs were adjusted for maternal weight only. NTD risks were obtained with fixed MSAFP cutoff of over 2.5 MoM. Down syndrome risks were calculated using MSAFP, MShCG, MSuE3 MoMs and maternal age with a term risk cutoff of 1 in 365. We analyzed our data for NTD and Down syndrome risks using LMP or US dating methods. We found that NTD risks increased from 3.7% by LMP to 6.4% by US dating among African-American (AA) women and from 3.6% by LMP to 8.5% by US among the European-American (EA) women. In contrast to the NTD risks observation we found that Down syndrome risks decreased from 12% by LMP to 7.5% by US among AA and from 9.3% by LMP to 6.2% by US dating among EA. These discrepancies may be related to the current practice of ultrasound estimation of gestational age from the size of the fetus. Babies with Down syndrome or neural tube defects are often small for gestational age (SGA). SGA babies affect maternal serum markers: MSuE3 is decreased, but MSAFP is increased. SGA may produce either an isolated decrease in MShCG or a combined increase in MShCG and MSAFP. Emerging methods of ultrasound diagnosis of SGA may improve the accuracy of prenatal marker screening.

Fluorescent restriction/PCR assay for *FRAXA* analysis among mentally retarded boys and girls. *F. Laroze*¹, *T. Billette de Villemeur*², *M. Bahuau*¹, *R. Couderc*¹, *D. Feldmann*¹, *J-P. Bonnefont*³, *C. Houdayer*¹. 1) Biochimie Biol. Moléculaire, Hôp. Trousseau, Paris, France; 2) Neuropédiatrie, Hôp. Trousseau, Paris, France; 3) Biochimie Génétique, Hôp. Necker Enfants-Malades, Paris, France.

Fragile X syndrome is the most common heritable genetic disease accounting for mental retardation and is usually related to the expansion of an unstable CGG repeat within the *FMR1* gene on the X chromosome. Expansion is associated with hypermethylation of the promoter region and subsequent downregulation of the gene. Despite averred robustness, laborious Southern blot-based diagnosis is not suitable for large-scale routine screening necessitated by neuro-pediatric practice. To meet these requirements, we optimized a previously described fluorescent PCR protocol, detecting all expansions in males and premutations in females, in adding a restriction step prior to amplification. In fully mutated females, failure of identification by PCR is accounted for by competition of the wild-type allele with the expanded one during the PCR process. Thus, genomic DNA was treated by methylation-sensitive restriction endonuclease *CfoI* which recognizes 3 cleavage sites on the PCR-targeted fragment. These sites in normal alleles are by and large unmethylated, which is probably also true in females despite random X inactivation. Therefore, cleavage of small wild-type alleles will prevent them to compete out full mutations in females during the PCR reaction. Furthermore, amplification from restricted and unrestricted genomic DNA in the same patient allowed semi-quantitative assessment of the methylation status at the *FRAXA* locus. A preliminary trial on 50 male and female DNA samples allowed us to ascertain the mutational status of all but one patient (a girl, for whom there is still diagnostic hesitancy), therefore leaving just one indication for Southern-blot. We believe this restriction/PCR strategy is a very powerful approach that dramatically reduces the recourse to Southern blotting, therefore allowing large-scale molecular screens in boys and girls with mental impairment of unknown etiology.

An anonymous screen of 6200 newborn boys for mutant FMR1 gene using bloodspot and a high-throughput PCR procedure in Taiwan. *C.C. Tzeng¹, S.J. Lin², C.H. Lin², L.P. Tsai³, W.L. Hwu⁴.* 1) Department of Pathology; 2) Department of Pediatrics, National Cheng-Kung University Hospital, Tainan; 3) Department of Pediatrics, Taipei Municipal Women and Children's Hospital, Taipei,; 4) Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan, R.O.C.

Fragile X syndrome (FXS), the most common cause of hereditary mental retardation, is caused by the expansion of CGG-triplet in the FMR1 gene on Xq27.3. All FXS patients inherited mutant gene from their mother. Identifying young carrier women to take preventive measures is thus rather practical. FXS is so far not treatable; however, early diagnosis followed by appropriate intervention will allow the affected children to achieve their highest potential. An effective large-scale screen of newborn boys could not only early diagnose FXS victims, but detect premutation carriers. Tracing family members of those carriers might further identify more young female carriers. In this study, we reported a high-throughput and inexpensive PCR procedure suitable for this purpose, and applied it to screen 6200 anonymous newborn boys to ascertain the prevalence of mutant FMR1 gene in Taiwan. DNA was isolated from bloodspot on filter paper by simply soaking in 5% Chelex-100 at RT for 20 minutes, followed by brief boiling. Optimal PCR relied on using a longer primer set and inclusion of 0.5M betaine to replace 7'-deaza-2'-dGTP. PCR products were then analyzed by separating on 5% polyacrylamide gel and staining with ethidium bromide, which could distinguish the normal allele at 10-CGG interval, as well as detecting premutation up to 98-CGG. Vast majority (98.4%) of the samples screened had CGG-triplet below 40, with remaining 98 between 40 and 52, 7 between 60 and 98, and 3 not measurable. The three subjects failed in PCR appeared most likely to be either FXS victim or carrier of large premutation. Our results implied that (1) the prevalence of mutant FMR1 gene in Taiwan would be one of every 620 X-chromosomes (10/6200), which is very similar to those reported from other populations; and (2) this high-throughout PCR procedure appears to be cost-effective and suitable for large-scale screen of newborn boys.

Evaluation of an automated commercially available PCR kit for determination of Fragile X (FRAXA) normal, gray-zone and premutation allele sizes. *N.M. Brown¹, M.J. Friez², J.W. Longshore², T.T. Stenzel¹*. 1) Department of Pathology, Duke University, Durham, NC; 2) Greenwood Genetic Center, Greenwood, SC.

Fragile X syndrome is the most common inherited form of mental retardation. The syndrome is characterized by moderate to severe mental retardation, macroorchidism, large ears, prominent jaw, and high-pitched jocular speech. This phenotype is associated with an expansion of the trinucleotide repeat (CGG)_n found in exon one of the FMR1 gene. Analysis of repeat length can be partially automated using a commercially available kit for performing polymerase chain reaction using a fluorescently labeled primer. We evaluated the PE Biosystems Fragile X Size Polymorphism Assay combined with a PE Biosystems ABI 310 capillary electrophoresis unit in a blinded study of twenty-four patient samples which had previously been sized by a modified Fu et al. (1991) protocol. The sample set contained fifteen samples that were normal/gray-zone (range 19 to 52 repeats) and nine with premutations (range 67 to 155 repeats). There was complete diagnostic agreement between the two assays as well as excellent correlation of allele sizes. The correlation coefficient for the normal/gray-zone and premutation samples was 0.998 and 0.988, respectively. Of the 29 normal/gray-zone alleles examined the average repeat difference between the two assays was 0.24 repeats per allele (7 repeats/29 alleles, range 0 to 2 repeats). Of the 9 premutation alleles examined the average repeat difference was 3.11 repeats per allele (28 repeats/9 alleles, range 0 to 7 repeats). These findings show an excellent correlation between an established method (modified Fu et al., 1991) for detecting normal, gray-zone and premutation alleles in the FMR1 gene and the PE Biosystems Fragile X Size Polymorphism Assay. The PE Biosystems Assay also provides a rapid size assessment without the use of radioactivity and reduces manpower needs.

Early diagnosis of neonatal citrullinemia using tandem mass spectroscopy. *D.N. Finegold¹, D.H. Chace², E.W. Naylor².* 1) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Neo Gen Screening, Inc., Pittsburgh, PA.

Neonatal citrullinemia or argininosuccinic acid synthase deficiency (ASD) is usually a severe urea cycle defect. ASD is included in the differential diagnosis of neonatal hyperammonemic coma. The symptoms, which result in a diagnosis, include increasing lethargy, difficulty feeding, vomiting, hypothermia, and hyperventilation. Often the diagnosis is initially missed, resulting in profound and prolonged hyperammonemia. Since most infants do not present until after 24 hours of life, they are frequently discharged to home before they become ill. Previously, newborn screening has been attempted for this disorder using a variety of filter paper blood and urine screening tests. None of these screening tests, however, have proved practical for mass screening. Tandem mass spectrometry (MS/MS) has recently been demonstrated to be a practical newborn screening method for a large number of inborn errors in amino acid, organic acid, and fatty acid metabolism. Using MS/MS, the basic amino acids can be rapidly and efficiently detected and quantified. We have used MS/MS to screen nearly 1 million newborns and have detected 4 newborns with acute neonatal and 1 with mild ASD. We present here a report on one of these patients. On the initial newborn blood specimen there was a significant elevation of citrulline. On recall at 8 days of age, the pediatrician evaluating the infant felt he was healthy, but quiet. Plasma ammonia was found to be 233 mmole/L. The infant was referred to Children's Hospital of Pittsburgh and within 12 hours the ammonia level had normalized. He was treated with a low protein diet and ammonia scavengers. He had 2 more episodes of hyperammonemia over the first year of life, which were aggressively treated, and then subsequently underwent orthotopic liver transplantation with resolution of all symptoms. He is currently developmentally normal at 2 years of age. We believe this is the first patient with ASD detected in the newborn period with MS/MS and successfully treated. We also believe this case demonstrates the value of MS/MS as a newborn screening method for the early detection of ASD.

Quality assurance practices in clinical biochemical genetics laboratories. *M.M. McGovern¹, M. Benach¹, S. Wallenstein¹, I. Lubin².* 1) Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY; 2) Centers for Disease Control, Atlanta, GA.

A nationwide survey of laboratories that offer biochemical genetic testing was conducted to determine the availability of testing, lab setting, personnel qualifications, and quality assurance (QA) and reporting practices in the areas of enzymology, substrate determination, and organic and amino acid analysis. In addition, policies regarding informed consent were studied. Data from the first 90 completed surveys was analyzed and revealed that hospital, research, and independent labs accounted for 65, 16, and 13% of testing labs respectively. All directors met the CLIA standard for director of a high complexity testing laboratory, although only 55% were ABMG certified in Biochemical Genetics. Most also serve as director for other types of non-genetic testing. Over 150 different analyses were being offered, with amino acid analysis the most common (82%). Most labs participate in one or more proficiency testing programs (95%) and are CLIA certified (96%). There was wide variation in the source of reference ranges and reagents, the methodologies used, and reporting practices for all types of analyses (i.e. only 63% of those offering amino acid analysis provide an interpretation of the results). Similarly, QA practices varied and were associated with a number of factors including laboratory setting and board certification of the director. For example, when a QA score was assigned for amino acid analysis based on responses to QA items derived from the ACMG Laboratory Practice Guidelines, ABMG certification of the director and a hospital setting were associated with a significantly better score. With regard to informed consent, only 19% of directors indicated a requirement for informed consent, although 45% were offering prenatal diagnosis, and 48% were offering carrier identification studies. Detailed analysis of the data is ongoing and will be presented. This data set will be of interest to the various professional organizations concerned with laboratory QA, credentialing of lab directors, and the clinical issues that accompany genetic testing.

Molecular testing for Fabry disease. *C. Wei, L. Han, L. Steele, J.T.R. Clarke, P.N. Ray.* Paediatric Laboratory Medicine, Hospital for Sick Children, Toronto, Ontario, Canada.

Fabry disease is an X-linked inborn error of glycosphingolipid metabolism resulting from deficient activity of the lysosomal hydrolase α -galactosidase A (α -Gal A) enzyme. The gene encoding α -Gal A, which contains seven exons and extends over 12 kb, is localized on the long arm of the X chromosome (band Xq22.1). More than 160 mutations including gene rearrangements, splice-junction defects, and point mutations, have been identified. There is no mutation hot spot and most mutations are confined to a single family. Molecular identification of a mutation in the proband makes it possible to offer precise carrier detection in female relatives. We have developed a strategy to detect mutations in the GLA gene. This assay consists of three stages: 1. Fluorescent PCR-based DNA fragment analysis to detect whole exon deletion or duplication, small deletions and insertions > 3 bp; 2. Heteroduplex analysis to detect single nucleotide change; and 3. Direct sequence analysis to confirm any potential mutations. In a preliminary study, we have identified 8 mutations in 9 Fabry families. These mutations include two nonsense, four missense, one splice site defect, one 2 bp deletion, and a nucleotide substitution in the 5'UTR. Among these mutations, four have not been previously reported. As molecular diagnostic testing becomes more and more important in patient management, direct analysis of the entire gene will be necessary to detect all mutations in genetic conditions lacking a mutation hot spot. Since sequencing the entire gene is costly and labor intensive, it is necessary to integrate a mutation screening method prior to sequencing in order to maintain the test sensitivity and reduce the cost. The development of a molecular diagnostic test for Fabry disease can serve as a model in the development of tests for other genetic conditions.aaa.

Frequency of new mutations and allele drop-out in Barth syndrome. *I.L. Gonzalez, J. Johnston, V. Funanage, R. Proujansky.* DuPont Hospital for Children, Wilmington, DE.

We have encountered the problem of allele drop-out during pcr amplification of the gene responsible for Barth syndrome (G4.5 or TAZ gene), whereby amplification of the mutated allele of some carrier females is either drastically reduced or abolished. Repeat amplifications on a single DNA may give mutation-positive, -negative, or uncertain results. Blood is the most common source of DNA for genetic testing and it is one of the tissues affected in Barth syndrome. It has been shown by others that carriers of Barth syndrome mutations exhibit skewed X-inactivation, which suggests that lymphocytes with the mutated G4.5 gene on the active X chromosome may be selected against. We hypothesize that methylation of the inactive X chromosome may interfere with amplification of the mutated G4.5 gene. It is also possible that the X chromosome carrying the mutated allele is preferentially eliminated (especially in older females). Two "remedies" have been tested to improve reliability of Barth syndrome carrier testing. The first is the use of LE-pcr ("linear-exponential" pcr) to amplify the 2 alleles from blood-derived DNA of potential carrier females. The second is to use buccal smear-derived DNA, as buccal epithelium is not a tissue affected by the syndrome and is not expected to exhibit skewed X-inactivation.

We have also found a surprisingly high frequency of new mutations in Barth families and have ascertained the origin of the mutation in families that are informative for linked polymorphisms. G4.5 mutations have originated de novo in mothers, and in both male and female grand- and great-grand-parents.

* LE-pcr consists of a linear multiplication stage followed by an exponential amplification stage. Protocol will be provided.

Success of DNA extraction by commercial kits from small volume bone marrow samples. *R. Aplenc^{1,2}, A. Deitz¹, A. Walker¹, J. Swoyer¹, K. Simbiri¹, B.J. Lange², M.A. Blackwood¹, T.R. Rebbeck¹.* 1) CCEB, University of Pennsylvania, Philadelphia, PA; 2) Department of Oncology, Children's Hospital of Philadelphia, Philadelphia, PA.

Little data exist on the quantity and quality of DNA extracted from small quantities of bone marrow by commercial DNA extraction kits. We compared the Qiagen DNA Blood Mini Kit and the Gentra Puregene DNA Extraction Kit in parallel extractions of bone marrow samples from 10 patients. The number of nucleated myeloid cells and platelet precursors were counted under a hemocytometer. Cell counts ranged from 12,500 to 610,000 cells per 5 mcl of bone marrow. DNA from 5 mcl aliquots of fresh marrow was extracted from each patient in duplicate using both kits. Additionally, 5 mcl aliquots of marrow were smeared on slides, air-dried, and extracted in duplicate. DNA concentration and 260/280 ratio was evaluated on all extractions using a spectrophotometer (Pharmacia Biotech GeneQuant II). All extractions were eluted in 50 mcl of proprietary elution solution. The mean Qiagen Blood Mini Kit yields on fresh and air-dried marrow were 22.4 mcg/ml (s.d. = 15.1 mcg/ml) and 9.1 mcg/ml (s.d. = 4.6). The Gentra kit mean yields on fresh and air-dried marrow were 48 mcg/ml (s.d. = 50.3 mcg/ml) and 17.1 mcg/ml (s.d. = 17.4 mcg/ml). The Gentra DNA yields on fresh and slide marrow specimens were significantly higher than the Qiagen DNA yields, $p = 0.0166$ (fresh) and $p = 0.0051$ (slide) by the Wilcoxon Matched Pairs Signed Ranks Test. Both kits yielded DNA with similar 260/280 ratios. The mean 260/280 for the Qiagen kit was 1.79 (s.d. = 0.10) and 1.788 (s.d. = 0.11) for the Gentra kit. Aliquots of all specimens were used in the CYP3A4 variant PCR RFLP assay with negative controls. Both kits amplified the CYP3A4 gene in all patients, both in fresh and slide specimens. These experiments indicate that commercial kits can be used to extract DNA from bone marrow aspirate slides. However in these parallel extractions the Gentra kit gave the highest DNA yields. Supported by a Bristol-Myers-Squib AACR Research Fellowship in Clinical Oncology Research.

Comparison of child-only versus mother/child sample collection in Fragile X testing. *K.M. Murphy, M.E. Nunes.*
USAF Medical Genetics Center, 81 MDG/SGOU, Keesler AFB, MS.

The USAF Medical Genetics Center provides genetic testing for Department of Defense health-care beneficiaries. Our laboratory performs 62% of all molecular diagnostic Fragile X tests for the 2 million spouses and children of active duty servicemembers (2.87 tests/10,000 beneficiaries/year). Because PCR-based Fragile X screening is less expensive and less time-consuming than Fragile X Southern analysis, two PCR-based testing protocols were compared. For the first protocol, patient samples were analyzed by PCR/PAGE/*GeneScan*. Maternal samples were requested when a mutation or premutation was found. For the second protocol, a patient and his/her mother's sample were both first analyzed by PCR. For both protocols, samples which failed to amplify and results which were uninformative, indeterminate, or revealed a premutation were further analyzed by Southern analysis. The rationale for testing mother/child pairs was to reduce the risk of missing a child mosaic for Fragile X expansion. In a two year period, 455 "child-only" samples (272 male, 183 female, 22 maternal) and 470 "mother/child" samples (363 male, 107 female, 457 maternal) were received. Four possible genetic diagnoses were detected: affected child, premutation child, X-chromosome aneuploidy, and premutation mother. Premutation mothers were scored only if diagnosed independent of an affected or premutation child. Chi-square analysis revealed no difference between the two test groups in detection of a genetic diagnosis overall (χ^2 is 0.8, $p = 0.37$), nor for each of the four diagnostic categories when analyzed separately (χ^2 is 1.06, $p = 0.79$). Separate analysis by gender also revealed no difference in rate of diagnosis detection between the two test protocols. Cost analysis for each protocol revealed \$3510 per genetic diagnosis for the "child-only" protocol and \$7962 per genetic diagnosis for the "mother/child" protocol. In contrast, if all diagnoses in both test populations could be detected by Southern analysis alone, the cost would be \$6751 per genetic diagnosis. PCR-based Fragile X testing of the proband alone provides cost-effective and efficient detection of genetic diagnoses except mosaicism.

Reverse dot-blot (RDB) hybridization as a new tool for molecular diagnosis of CAH due to 21-hydroxylase deficiency. *Y. Yang, N. Corley, J. Garcia-Heras.* Genetic Testing Center, Texas Dept. of Health, Denton, TX.

Congenital Adrenal Hyperplasia (CAH) due to 21-hydroxylase deficiency is an autosomal recessive disease causing over 90% of all the CAH cases. The classic type of CAH has an incidence of about 1/10,000 to 1/15,000, while the nonclassic type has a much higher frequency. The detection of gene deletions, large scale gene conversions, the 8 bp deletion in exon 3 and a panel of nine most common mutations (P30L, AC656->G, I172N, I236N-V237E-M239K, V281L, L307insT, Q318X, R356W, P453S) in 21-hydroxylase gene (CYP21) comprises about 90% of the mutations associated with CAH. Currently allele-specific PCR and allele-specific oligonucleotide (ASO) hybridization are commonly used to detect the whole or part of the nine-mutation panel. In spite of their accuracy, these methods can be tedious and labor intensive because for each mutation site, two allele-specific PCR reactions or two ASO hybridizations are needed to detect normal and mutant sequences.

We have developed a reverse dot-blot (RDB) system, in which the process is the reverse of the traditional ASO hybridization, to simultaneously detect both normal and mutant sequences at the nine common mutation sites. In this method, oligonucleotides representing both normal and mutant sequences of the nine mutations were spotted on one strip of nylon membrane. The ten exons of the CYP21 gene from a test sample were amplified by multiplex PCR, labeled by biotin and used as hybridization probes. The detection of the mutations was done in only one hybridization, followed by chemiluminescence signal detection. We identified mutations causing CAH in 30 patients ascertained by The Texas Newborn Screening Program and confirmed the results by DNA sequencing. Like previous RDB studies of β -thalassemia and cystic fibrosis, which are also associated with a large number of mutations, this first report of applying RDB to CAH tests demonstrates that RDB is more efficient and simpler than other molecular methods. For this reason, and its potential for automation, RDB should become a procedure of choice for routine confirmation of CAH cases in large newborn screening programs like the Texas Newborn Screening Program.

Quantitative real-time PCR: a new method to determine the copy number of sex determining genes. *L.P. Tsai^{1,3}, H.H. Lee², S. Ching³, Y.-H. Zhang⁴, E. Vilain^{3,4}*. 1) Dept Pediatrics, Taipei Mun Womens/Child Hosp, Taipei, Taiwan; 2) Dept Biological Chemistry; 3) Dept Human Genetics; 4) Dept Pediatrics, UCLA, Los Angeles, CA.

Counting the number of gene copies traditionally has been a challenge for PCR-based molecular diagnoses. Gene duplication has been recently recognized to be a frequent molecular mechanism for sex reversal (XX males and XY females). Duplication of a 160kb region on Xp21.3, containing the DAX1 gene, is responsible for XY females with dosage sensitive sex reversal(DSS). Duplication of a fragment of chromosome 17 including the gene SOX9 was shown to lead to DSS with XX males. The purpose of our investigations was to provide a rapid and accurate method for quantitation of the number of gene copies that could be applied to the diagnosis of DSS. Real-time quantitative PCR(TaqMan) has been shown to be accurate in quantifying gene expression after reverse transcription. It is based on the cleavage of fluorescent dye-labeled probes by the 5'-3' exonuclease activity of the Taq polymerase, and the measurement of fluorescence intensity during PCR. We have used TaqMan to detect the number of copies of the X-linked gene DAX1 per diploid human cell genome. Insl-3, an autosomal gene, was used as a control. DAX1 and Insl-3 were labeled with FAM and VIC dyes, respectively, and the CT(threshold cycle) value measured during the PCR process. The relative DAX1 copy number was calculated by measuring the DAX1/Insl-3 copy ratio. On three genomic templates, 46,XY, 46,XX and 49,XXXXY, the relative DAX1 copy number was quantified at approximately 1:2:4, respectively. DNA from an XY female patient with a known duplication of DAX1 was assayed. The relative DAX1 copy number was 2, confirming the presence of a double dose of DAX1. We have performed an efficient and rapid measurement of DAX1 copies on genomic DNA. This new method provides a reliable alternative to the Southern blot, poorly reproducible technique for quantitation. TaqMan can be applied to the DNA quantitation of any gene and will prove useful as a rapid diagnostic tool for the molecular evaluation of patients born with ambiguous genitalia or possible sex reversal.

Mutation analysis of the entire mitochondrial DNA using Denaturing High Performance Liquid

Chromatography (DHPLC). *H. Smeets¹, B. Van den Bosch¹, J. Nijland¹, H. Scholte², C. DeDie¹, R. Van den Bogaard³, M. De Visser³, I. De Co².* 1) Dept Molecular, Cell Biol & Gen, Univ Maastricht, Maastricht, Netherlands; 2) Depts Child Neurology & Biochemistry, Erasmus Univ, Rotterdam, Netherlands; 3) Depts Clin Genet & Neurol, AMC, Amsterdam, Netherlands.

In patients with mitochondrial disease a continuously increasing number of mtDNA mutations and polymorphisms have been identified. Most pathogenic mtDNA mutations are heteroplasmic, resulting in heteroduplexes after PCR amplification of mtDNA. To detect these heteroduplexes, we used the technique of Denaturing High Performance Liquid Chromatography (DHPLC). The entire mtDNA was amplified in 13 fragments of 1-2 kb, digested in fragments of 90-600 bp and resolved at their optimal melting temperature. The sensitivity of the DHPLC system was high with a lowest detectable percentage of 0.5% for the A8344G mutation. The entire muscle mtDNA from 6 patients with mitochondrial disease was screened and 3 mutations were identified. The first patient with a limb-girdle-type myopathy carried an A3302G substitution in the tRNA-Leu(UUR) gene (70% heteroplasmy), the second patient with mitochondrial myopathy and cardiomyopathy a T3271C mutation in the tRNA-Leu(UUR) gene (80% heteroplasmy) and the third patient with Leigh syndrome a T9176C mutation in the ATPase6 gene (93% heteroplasmy). We conclude that DHPLC analysis is a sensitive and specific method to detect heteroplasmic mtDNA mutations. The entire automatic procedure can be completed within 2 days and can also be applied to exclude mtDNA involvement, providing a basis for subsequent investigation of nuclear genes. Subsequently, we optimised the procedure at the single cell level to test for heteroplasmy in single oocytes and embryos to determine the natural mutation load of mtDNA and the possible presence of paternal mtDNA.

The presence of the late onset Tay-Sachs disease mutation Gly269Ser in two Brazilian cousins affected by early onset Tay-Sachs disease. *R. Rozenberg¹, A.M. Martins², L.V. Pereira¹.* 1) Depto de Biologia, IB-USP, São Paulo, SP, Brazil;; 2) Escola Paulista de Medicina, Unifesp, São Paulo, SP, Brazil.

Tay-Sachs disease (TSD) is an autosomal recessive disease of lysosomal storage, characterized by progressive neurologic degeneration. Children affected by classical TSD manifest first symptoms around 6 months and die before the age of 5. TSD is caused by mutations in the HEXA gene, coding for the a subunit of the enzyme hexosaminidase A. Late onset TSD is a rare variant phenotype with appearance of first symptoms during the second or third decade of life. The Gly269Ser mutation, found in 2% of Ashkenazi Jewish TSD carriers, is known to cause the late onset phenotype when in combination with early onset mutations. We describe a family with two affected cousins, a boy and a girl, whose mothers are sisters. The fathers and both couples are non consanguineous. The family denies any Jewish or Portuguese ancestry. The boy and the girl, currently 2.5 and 4 years old, showed the first symptoms of TSD with 6 and 10 months, respectively. Both are confined to bed, presenting hypereflexy, hypotonia and no communication. The girl was diagnosed with TSD variant B1 (TSD-B1) after testing with 4-MUG and 4-MUGS substrates. The patients and their parents were tested for the 3 most common Ashkenazi Jewish (AJ) mutations (1278insTATC, IVS12+1, Gly269Ser) and for the DN allele (Arg178His), a common TSD-B1 causing mutation. The boy carries the Gly269Ser mutation inherited from his mother and another unidentified mutation. The girl was shown to be a compound heterozygote of mutations Gly269Ser and Arg178His, inherited from his mother and father, respectively. None of the individuals carry the other two AJ mutations. The presence of the late onset mutation Gly269Ser in these two affected children is an unexpected finding since the children present early onset TSD. Although the possibility that another mutation besides the Gly269Ser is causing the disease in these children must be excluded, these results suggest a dominant negative effect of the other mutations over the Gly269Ser mutation. Moreover, they indicate that the Gly269Ser mutation may be present in children with early onset TSD.

Dystrophin duplications: distribution, clinical significance, and somatic mosaicism. *S. Abbs¹, T. Smith¹, M. MacLeod¹, M. Bobrow², C. Mathew¹, F. Muntoni³, S.C. Yau¹.* 1) Genetics Centre, Guy's Hospital, London, UK; 2) Dept of Medical Genetics, Addenbrooke's Hospital, Cambridge, UK; 3) Imperial College, Hammersmith Hospital, London, UK.

Quantitative fluorescent multiplex PCR analysis was used to screen for dystrophin duplications in 104 Xp21 muscular dystrophy patients who had previously tested negative for deletion mutations. 38 duplications were identified (37%), significantly more than the 17% in published data ($p < 0.0001$). The effect of the duplication on the reading frame could be predicted in 27 cases, allowing genotype-phenotype correlations. Only 20 (78%) cases complied with the reading frame hypothesis, compared with 92% described for dystrophin deletions. Deletions are more common in the distal region of the gene (proximal:distal ratio = 1:3.5), whereas duplications are more common in the proximal region (3.3:1). Since most deletion mutations which disagree with the reading frame hypothesis are proximal, and given the high frequency of proximal duplications, this explains why more duplications disagree with the hypothesis. The six exceptions comprised 3 out-of-frame duplications (exons 2, 2-4, and 2-43) associated with milder than expected phenotypes, and 3 in-frame duplications (2-7, 3-5, and 3-13-) causing severe phenotypes. One DMD patient exhibited somatic mosaicism for an out-of-frame duplication of exon 51, which was present in only 50% of lymphocytes. However, a muscle biopsy showed no dystrophin, consistent with the observed DMD phenotype, suggesting that there are more mutant cells in muscle than in lymphocytes. A 13bp sequence flanked the duplication breakpoint, suggesting the likely mutational mechanism to be unequal recombination between sister chromatids. To our knowledge this is the first report of DMD being caused by somatic mosaicism for a duplication, adding to previous reports with deletions and point mutations, and demonstrating that the full spectrum of dystrophin mutations can arise at any time in development and give rise to muscular dystrophy.

Mutations Screening in PKD1 transcript, using RT-PCR specific primers. *S. Burtey*¹, *A.M. Lossi*¹, *Y. Berland*², *M. Fontes*¹. 1) INSEM U 491. Medical Genetics, Fac de medecine, Marseille, France; 2) Departement de Nephrologie. Hopital de Ste Marguerite. 271 Bd. de Ste Marguerite. 13009 Marseille. France.

Autosomal dominant polycystic kidney disease is a common disorder, primary characterized by progressive renal enlargement and cyst formation, leading, in the majority of patients, to end-stage renal failure. Cysts formation has also been observed in liver and pancreas. Cerebral-artery aneurism has also been reported. This disorder is genetically heterogenous, but about 85% of cases are due to a mutation in the PKD1 gene, located on the short arm of chromosome 16 (16p13.3). This gene spans about 50 kb of genomic DNA, is composed of 46 exons, and the ORF is of 13 kb. The incidence of PKD1 in the population is about 1/1000 individuals, making of this disorder the most frequent genetic disorder in human, however, mutations in the gene has not been extensively reported, and routine clinical diagnostic by molecular analysis not largely developped. This situation can be explained by the fact that 70% of the coding sequence is present as highly homologous copies in transcribed sequences located in more centromeric regions of the short arm of chromosome 16. In order to overcome these problems, and to propose a reliable diagnostic, we have designed primers from data bases sequences, spanning regions in which we have detected slight differences between PKD1 and its homologue. We have used these primers to amplify RT-PCR products, ranging from 1 to 3 kb, and showed that there were PKD1 specific. We used this test to screen for mutations in 8 polykystic patients, with a clinical presentation evoking PKD1, with or without a familial history. Surprisingly, we found 12 polymorphisms, and 4 mutations. This is the first strategy to screen mutations over all the coding region of the gene, by a quick and reliable method.

Determination of the carrier frequency of the 946delGAG mutation in the DYT1 gene in Ashkenazi Jewish population using non-radioactive allele-specific oligonucleotide hybridization. *J. Dong¹, D.R. Katz¹, R. Kornreich¹, R.J. Desnick¹, M.F. Brin², C.M. Eng¹.* 1) Department of Human Genetics; 2) Department of Neurology, Mt Sinai Sch Medicine, New York, NY.

Early-onset idiopathic torsion dystonia (ITD) begins at or before age 28 with abnormal involuntary movements. The disease is autosomal dominant with approximately 30% penetrance as estimated from clinical studies. DYT1, the gene for one form of early-onset ITD, is located on chromosome 9q34 and encodes the putative ATP-binding protein, torsinA. A GAG deletion in DYT1 is responsible for nearly all cases of early-onset ITD in Ashkenazi Jewish (AJ) and non-AJ patients. Haplotype analyses showed that >90% of AJ individuals with this mutation share a common haplotype; spontaneous mutations have been reported rarely. Although clinical studies suggested that the gene frequency in the AJ population should be 1/2000 - 1/6000, no DNA based screening has been reported. We have determined the carrier frequency of this mutation in 2000 anonymous DNA samples collected with informed consent from AJ individuals in the New York Metropolitan area. Using a non-radioactive allele-specific oligonucleotide (ASO) hybridization assay, we found the frequency of the 946delGAG mutation in this population was 5/2000 (95% C.I., 1/172-1/909). Since the disease frequency of ITD in the Ashkenazi population was estimated to be 1/15000 to 1/23000 (Risch, N et al 1995. *Nature Genet* 9:152-158), our results imply a population-based penetrance of ~1.7- 2.7%. This apparent discrepancy in gene frequency and observed disease frequency may be due to the presence of modifying factors such as gene-gene interactions that may lead to decreased or increased penetrance, as has been observed in several large pedigrees. Alternatively, it may define a subset of individuals with late-onset or milder symptoms. Thus, the detection of this mutation can facilitate accurate diagnosis and genetic counseling for families and individuals affected with early-onset ITD, and provides further basis for identifying factors that modify clinical expression of the disease.

Identification of point mutations in the dystrophin gene: diagnostic and therapeutic relevance. *S. Chambert, C. Saquet, M. Claustres, S. Tuffery-Giraud.* Lab Genetique Moleculaire, Montpellier, France.

Alternative strategies to cDNA-based gene therapy have been recently described in Duchenne (DMD) and Becker (BMD) muscular dystrophies consisting in either use of aminoglycosides to promote translation read-through of nonsense codons or use of antisense nucleotide strategy to modulate splicing of endogeneous mRNA transcript. If the correction of point mutations were to become a feasible treatment for DMD, then it would be important that the mutations will be identified in as many patients as possible. One third of DMD cases with no gross deletions presents point mutations in the coding region of the gene that usually lead either to splicing errors or premature stops. The identification of the underlying gene defect have not been determined in the majority of these patients as the detection of point mutations requires more extensive analyses than large gene defects, which are easily detected by routine PCR technologies. Our group is focused on the detection of point mutations in the dystrophin gene. We have established a mutation detection method based on RNA and the scanning of the whole encoding 11-kb region using protein truncation test (PTT), instead of the analysis of a limited number of exons at the genomic level. We have tested 50 patients originated from anywhere in France and found mutations in 86% of them; the analysis is currently in progress in a group of 20 additional patients. The identified mutations of which 80% were novel included 33% nonsense mutations, 28% splicing mutations, 25% frameshift mutations, and 14% small deletions or duplications. The exhaustive analysis of the dystrophin transcripts in these patients allowed to demonstrate that most splice-site mutations have complex effects on transcript structure and that some nonsense mutations are associated with exon skipping. Besides the diagnostic significance of identifying the causative mutation in sporadic cases and intragenic recombinants that are refractory to indirect diagnostic methods, the identification of point mutations in the dystrophin gene may be relevant for therapeutic purposes. Supported by grants from Association Francaise contre les myopathies (AFM).

Low level somatic mosaicism; an issue in molecular diagnosis of tuberous sclerosis (TSC). *A.C. Jones, J.R. Sampson, J. Maynard, J. Cheadle.* Inst Medical Genetics, UWCM, Cardiff, Wales.

Genetic mosaicism, both somatic and gonadal, has been reported in a number of Mendelian disorders that exhibit a high frequency of new mutations. Furthermore, patients mosaic for pathogenic mutations may have atypically mild phenotypes, making clinical determination of disease status and subsequent genetic counselling problematic. Somatic mosaicism has been estimated to account for approximately 10% of all de novo mutations in tuberous sclerosis (TSC) (OMIM 191100 and 191092) and could result in failure of molecular diagnosis because of an inability to detect mutant alleles present at low frequency. Here we report the identification of aberrant exonic dHPLC profiles in three mildly affected TSC cases. Despite the aberrant profiles, both automated and manual sequencing of the exons involved proved normal. In these three cases the intensity of the mutant profile was reduced in comparison to wild-type, suggesting under representation of the mutant allele. Amplification products of each of the exons associated with an aberrant dHPLC profile were cloned and sequenced identifying the likely pathogenic mutations 2724-1G®C in TSC1 and 1462-28 Del 42bp and 1772-1774 Del 4bp in TSC2. The mutant TSC2 alleles accounted for 18/104 (17%) and 7/93 (7%) clones characterised. The proportion of mutant TSC1 alleles is still to be accounted for.

Fluorescence-based homogeneous assays for rapid detection of human gene mutations. *X. Hu¹, B. Belachew², L. Chen¹, J. Zhang¹, P. Pingerelli², D. DuBois².* 1) Dept. of Molecular Medicine, Stratagene Corp, La Jolla, CA; 2) Stratagene Corp, Cedar Creek, TX.

The unique hairpin conformation of fluorescently labeled molecular beacons enables them to distinguish mutations in DNA sequence that differ by a single nucleotide. Using molecular beacon technology, a series of single-tube homogeneous assays for rapid detection of common human gene mutations have been developed (below). Each assay was developed using two allele-specific molecular beacons, genotype-specific DNA controls and target-specific PCR primers. Such single-tube assays offer several advantages over existing mutation scoring techniques: First, hairpin-shaped molecular beacon probes are more specific in distinguishing single base pair mismatches than linear probes. Second, the test is performed in a closed tube and no post-PCR manipulation of samples is required; hence, the user saves time and effort and significantly reduces the risk of PCR product carry-over contamination. Third, the capability of using two allele-specific molecular beacons in the same PCR solution enables simultaneous determination of three possible genotype representations (two homozygotes and a heterozygote) of two allelic variants in target DNA. Additionally, It definitively discriminates a true negative result from a false negative result due to PCR failure. Finally,, the molecular beacon technology can be adapted for high-throughput assays. Currently, with 96-well PCR plates, it takes about 4 hours to complete screening of 96 DNA samples. The assay throughput can be further enhanced by the use of higher density plate format, e.g., 384-well format. Single-tube assay kits: CCR2-64I, CCR5-del32, SDF-1 (3'-A), Factor V Leiden, Factor II (G20210A), MTHFR (C677T), HFE (C282Y), CFTR (del508), CYP2D6*4 (G1934A), CYP2C19*2 (G681A), CYP2C19*3 (G636A), NAT2 (C481T), NAT2 (G590A).

***CFTR* mutation detection in African American and Hispanic individuals with cystic fibrosis (CF).** *R.A. Heim, B.A. Allitto.* Genzyme Genetics, Framingham, MA.

Characterizing *CFTR* mutations in minority populations is difficult given sample availability and limitations to assay efficiencies. We determined mutation detection rates in unrelated CF patients of African American and Hispanic ancestry using our diagnostic sample set, referred from throughout the U.S., and an assay that tests 70 *CFTR* mutations. The analysis consisted of multiplex amplification and a pooled ASO hybridization strategy, followed by specific mutation identification by hybridization with individual ASOs.

Among 292 African American CF chromosomes tested for 70 *CFTR* mutations, a total of 26 different mutations were detected on 164 chromosomes. DF508 accounted for 28.1% of the chromosomes and 3120+1G>A accounted for 12.7%. The unexpectedly low frequency of DF508 may represent the non-referral of CF patients with identified DF508 mutations to our diagnostic laboratory. Five mutations (A559T, 1812-1G>A, G551D, S549N, and 3791delC) were present at a frequency of 1-2%. All others were present at <1% frequency. Of the 26 mutations identified, only 7 are considered to be specific to African Americans. 336 CF chromosomes from individuals who identified themselves as Hispanic but whose origin is unknown, were also tested for 70 *CFTR* mutations. A total of 28 different mutations were identified on 198 chromosomes. DF508 accounted for 36.3 % of the chromosomes and G542X for 2.7%. Nine mutations (N1303K, DI507, R334W, S549N, R1162X, I148T, W1089X, and 3120+1G>A) had a frequency of 1-2%. All others were present at <1% frequency. Of the 28 mutations identified in Hispanics, 15 are represented in the African American subset of 26, and two are considered to be specific to African Americans.

In summary, overall detection rates were 56% in African Americans and 59% in Hispanics. Since an unknown proportion of samples positive for a common mutation are not sent to a reference laboratory, these detection rates are conservative. A total of 39 mutations were identified in these two ethnic groups, suggesting that for clinical laboratories serving the general population a comprehensive mutation panel may be clinically relevant.

Molecular diagnosis of Korean hemophilia A patients by direct analysis of inversions and deletions in factor VIII gene. *J. Han*¹, *K.H. Kim*¹, *I.H. Kim*², *J.N. Lee*³, *S.Y. Lee*⁴, *I.J. Kim*⁵, *C.M. Kim*⁵. 1) Clinical Pathology, Col Med, Dong-A Univ, Pusan, Korea; 2) Biochemistry, Dong-A University College of Medicine; 3) Clinical Pathology, Pusan Paik Hospital; 4) Pediatrics, College of Medicine, Inje University; 5) Biochemistry, College of Medicine, Pusan National University.

Hemophilia A is the commonest X-linked bleeding disorder with an incidence of 1/5000 Caucasian males. Mutation analysis of factor VIII gene is complicated by its large size and frequent de novo mutations. DNA-based diagnosis of hemophilia A has previously been carried out only by linkage analysis in Korea. We aimed to establish direct mutation detection in Korean hemophilia A families. DNA was extracted from the blood of 107 unrelated hemophilia A patients. The inversion was detected by a single-tube PCR assay using four primers differentiating the wild type, inversion, and carrier. For gross gene deletion, PCR with exon-specific primers was performed. In 20(18.7%) patients, an inversion was found. The deletion of one or more exons in factor VIII gene was detected in two(1.9%) patients. Direct analysis successfully detected mutations in 22(20.6%) of 107 patients. This is the first report on direct analysis of factor VIII gene mutation in Korean hemophilia A patients and provides improved diagnostic tool for carrier detection and prenatal diagnosis.

Detection of heterozygous *SMN1* deletions in SMA families using a simple fluorescent multiplex PCR method. P.

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Spinal muscular atrophy (SMA), which represents one of the most common fatal autosomal recessive disorders, results in most cases from homozygous deletions of the telomeric copy of the survival motor neuron gene (*SMN1*). Duplication of the *SMA* locus complicates the detection of SMA carriers, which frequency has been estimated to 1/40, and this limits the efficiency of genetic counseling in affected families. To facilitate the detection of SMA carriers harboring a heterozygous *SMN1* deletion, we developed a multiplex PCR assay of fluorescent fragments. We simultaneously amplified exons 7 of the *SMN1* and *SMN2* genes using a mismatch primer which introduced a *Dra*I restriction site into amplified *SMN1* exon 7, *BRCA1* exon 11, and *MLH1* exon 18 which contains a natural internal *Dra*I restriction site. After electrophoresis on an automated sequencer, each multiplex PCR yielded a pattern composed of four fluorescent peaks corresponding to exonic fragments of *BRCA1*, *MLH1*, *SMN1* and *SMN2*, respectively, and electrophoregrams generated from different samples were superimposed. We tested 86 parents of SMA patients carrying a homozygous *SMN1* deletion. An approximately 0.5 reduction of the *SMN1* peak area, indicative of a heterozygous deletion, was clearly observed in 80 parents (93%). Two *SMN1* copies were detected in six putative carriers. In 4 out of these 6 families, we were able to demonstrate the existence of 2 *de novo* deletions and 2 *SMN1* duplications. Although this assay, like other previously published methods, cannot detect small intragenic *SMN1* mutations and heterozygous *SMN1* deletions in the presence of a *SMN1* duplication on the other chromosome, it should facilitate genetic counseling, limiting therefore prenatal screening in relatives of SMA patients, and the detection of SMA patients harboring a heterozygous *SMN1* deletion, who must be screened for *SMN1* small intragenic mutations on the other allele.

Development of a rapid molecular diagnostic assay for carrier screening for Canavan disease. *T.L. Stockley¹, A. Feigenbaum², T. Brutzki¹, J.T.R. Clarke², P.N. Ray¹*. 1) Department of Paediatric Laboratory Medicine; 2) Department of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, ON, Canada.

Canavan disease is a severe neurodegenerative autosomal recessive disorder caused by deficiency of the enzyme aspartoacylase (ASPA). Deficiency of ASPA causes accumulation of high levels of N-acetyl aspartic acid (NAA) in the CNS resulting in spongy degeneration of the brain, mental retardation and hypotonia. Symptoms of Canavan disease begin at 3-5 months of age with death in childhood.

Canavan disease is most frequent in the Ashkenazi Jewish population. Two previous population studies have indicated that the carrier frequency for Canavan disease among Ashkenazi Jewish individuals is approximately 1 in 40, which is similar to the carrier frequency for Tay-Sachs disease (1 in 30). However, the low incidence of Canavan disease reported from Canadian centres suggests that either the carrier rate for Canavan is lower in Canadian Ashkenazi Jewish or that children affected with Canavan disease are possibly being misdiagnosed. Molecular analysis of carriers of Canavan mutations in the Canadian Ashkenazi Jewish population is required in order to investigate this discrepancy.

We have developed a rapid molecular diagnostic test for three common ASPA mutations using a fluorescent allele specific PCR technique that is ideally suited to population screening. This assay is being used in a large scale population screening study to determine the actual frequency of Canavan carriers in the Canadian Ashkenazi Jewish population, and to assess the necessity of ongoing carrier screening. The new molecular assay for Canavan mutation analysis and the results to date from the population screening will be presented.

Genotyping Disease-Associated SNPs with Real Time PCR and FRET Chemistry: An Alternative Diagnostic Tool to Standard PCR-RFLP Methods. *S.B. Parks¹, R. Press^{1,2}, B.W. Popovich¹*. 1) Dept. of Molecular & Medical Genetics; 2) Dept. of Pathology, Oregon Health Sciences Univ., Portland, OR.

In an effort to improve efficiency, reduce costs, and decrease turn around time for common DNA diagnostic assays, we have investigated the use of real-time PCR coupled with fluorescence resonance energy transfer (FRET) hybridization probes for genotyping single nucleotide polymorphisms (SNPs). We have evaluated FRET-based assays for analysis of the hemochromatosis C282Y and H63D mutations, as well as the factor V Leiden, and prothrombin G20210A mutations using LightCycler (Roche) technology. Clinical validation of each assay was accomplished through the parallel analysis of 150 patient specimens by both the FRET probes assay and the standard RFLP method. For each of the above mutations we found the FRET genotypes to be in 100% concordance with the results generated by PCR-RFLP, which has been the recognized standard for genotyping these targets. In addition to genotype accuracy, we have compared the FRET-based assay to the standard RFLP method with respect to labor and reagent costs, genotype interpretation, and turn around time. Comparative data will be presented on each of these parameters. We have found that FRET hybridization probes provide an accurate, semi-automated and efficient means of genotyping single nucleotide polymorphisms. Furthermore, we have evaluated both manufactured analyte specific reagents (ASRs) as well as homebrew assays for FRET based genotyping, and will present both performance and fiscal data comparing these reagents. Based on these data, the OHSU DNA Diagnostic Laboratory has implemented real time PCR/FRET assays as the standard operating procedures for the diagnosis of hemochromatosis C282Y and H63D, factor V Leiden, and prothrombin G20210A mutations.

RECURRENT LGMD2A (CALPAINOPATHY) MUTATIONS IN BRAZILIAN PATIENTS. *F. Paula¹, E.S. Moreira¹, A.L.F. Bernardino¹, A. Kai¹, M.R. Passos-Bueno¹, M. Vainzof^{1,2}, M. Zatz¹.* 1) Center for the Study of the Human Genome, Universidade de S. Paulo, S. Paulo, Brazil; 2) Dept. Neurologia, FMUSP, Universidade S.Paulo, Brazil.

LGMD2A, an autosomal recessive form of limb-girdle muscular dystrophy, is caused by mutations in the calpain-3 (CAPN3) gene. The clinical course shows great inter and intrafamilial variability. About 97 distinct pathogenic mutations have been identified along the calpain gene. We analyzed the CAPN3 gene, which has 24 exons, in patients from 22 unrelated LGMD2A Brazilian families (8 screened through linkage and 14 through calpain deficiency in muscle). These families include 67 patients. In the first ten patients, screened for the entire gene, 13 mutations were found in four exons: 2, 11, 21 and 22. We analyzed then 12 additional families only for these exons and found mutations in 6 of them. In 14 of the 22 families, we identified 24 mutated alleles: in exon 2, the R110X mutation in 5 patients (3 in homozygosity); in exon 11, the R490W and G496R mutations in 2 patients (both in heterozygosity); in exon 21, the R748Q (in one allele) and a novel 2243-2244insT mutation (in homozygosity) in 2 patients; in exon 22, the R769Q in one and the 2362AG->TCATCT change in 5 (4 in homozygosity) patients. The exon 21 was previously described as a hot spot of mutations and exon 11 as carrying mostly missense mutations. The R110X mutation (found in 5 patients) has apparently been described only in Brazilian families while the 2362AG->TCATCT mutation (found also in 5 patients) is prevalent in Guipzoa-Basque Spain. The origin of the recurrent mutations is currently under investigation. This study showed that: a) null mutations were associated with total absence of calpain and missense mutations with calpain deficiency but no direct correlation was observed between the type of mutation and clinical course, in particular due to intrafamilial variability; b) Although our population is highly miscigenated about 50% of mutations seem to be concentrated in only 4 exons within the CAPN3 gene in Brazilian LGMD2A patients. Supported by FAPESP, CNPq, IAEA and PRONEX.

Referral testing for cystic fibrosis. *V.M. Pratt, T. Brown, L. Kam-Morgan, M. Eisenberg, G.C. Heyward, S.M. Anderson.* Laboratory Corporation of America, Research Triangle Park, NC.

Cystic fibrosis (CF) is one of the most common autosomal recessive disorders with an incidence in Caucasians of approximately 1/2500; the incidence is variable in other ethnic populations. Typical symptoms include pulmonary obstruction, chronic infections, pancreatic insufficiency, meconium ileus, failure to thrive, and infertility. Testing should be considered for symptomatic patients, positive family history, or suggestive ultrasound findings. The NIH issued a consensus statement recommending carrier screening for all pregnant couples and those considering pregnancy [1997]. Over 800 CF mutations have been identified. During a 17-month period we have performed mutation screening for 31 mutations using the Perkin-Elmer (PE) oligonucleotide ligation assay kit. In 8499 specimens received from throughout the country, we detected 32 affected individuals, 492 carriers, and 7975 negatives. We referred 92 (1.1%) specimens for additional mutation testing based on possible affected status or close family history. Four (4.3%) had mutations not in our PE panel: 1 clinically affected Northern European Caucasian (DF508/3120+1G®A), 1 clinically affected Northern European Caucasian (DF508/Y563D), 1 African American with a reported CF carrier mother (3120+1G®A/-), and 1 Iranian with a reported CF carrier brother for I148T (I148T/-). Interestingly, the 3120+1G®A has a frequency of 12.2% in African-American CF patients. In conclusion, the PE CF screening panel detects the majority of Caucasian mutations. Adding mutations such as 3120+1G®A would improve the assay because of the incidence in non-Caucasian populations. We recommend additional mutation screening to rule out CF especially when one mutation is identified or if there is a close family history when the mutation is unknown.

Comparative sequence analysis (CSA) facilitates detection of mutations in the tuberous sclerosis genes TSC1 and TSC2. *J.R. Yates^{1, 2}, K.A. Diver³, D.J. Valler³, T. Bedenham³, C.J. Mattocks³, J.L. Whittaker³.* 1) Department of Medical Genetics, University of Cambridge, Cambridge, UK; 2) Department of Medical Genetics, Addenbrooke's Hospital, Cambridge, UK; 3) Molecular Genetics Laboratory, Addenbrooke's Hospital, Cambridge, UK.

Tuberous sclerosis (TSC) can be caused by mutations in either the TSC1 gene on chromosome 9q34 or the TSC2 gene on chromosome 16p13.3. The TSC1 gene spans 40 kb with 21 coding exons, the largest being exons 15 (559 bp) and 23 (517 bp of coding sequence) and the remainder varying in size from 44 to 188 bp. The mutations are diverse and distributed throughout the gene. Almost all reported mutations would be predicted to result in a truncated protein. Large scale rearrangements are rare. The TSC2 gene spans approximately 45 kb of genomic DNA and comprises one exon of 488 bp and 40 smaller exons of 49 - 213 bp. The mutation spectrum includes large deletions spanning part or all of the gene and diverse small mutations with few observed in more than one family. Truncating mutations are common but missense mutations also occur. The identification of mutations in the TSC genes is a major challenge for laboratories undertaking mutation screening as a diagnostic service. Comparative sequence analysis (CSA) is a modification of automated fluorescent sequencing which employs a novel data-handling technique to present the output in a visual format that allows the rapid identification of mutations. We have compared CSA with our existing method of screening all coding exons of TSC1 and TSC2 by conformation sensitive gel electrophoresis (CSGE) followed by conventional automated sequence analysis. CSA proved much faster with a comparable sensitivity. It was particularly effective in reducing the time taken for the interpretation of point mutations.

Quantitative analysis of the SMN1 gene in SMA: application for diagnosis of affected cases without homozygous deletion and for carrier and prenatal diagnoses. *E.F. Tizzano, I. Cusco, M.J. Barcelo, M. Baiget.* Genetics, Hospital Sant Pau, Barcelona, Spain.

Approximately 90% of the autosomal recessive spinal muscular atrophy (SMA) cases are caused by homozygous deletions in the Survival Motor Neuron gene (SMN1) located at 5q13. In this work, we validated in our Spanish population a recently reported quantitative method for analysis of the SMN1 gene to perform molecular diagnosis of SMA in symptomatic cases without homozygous deletion of the SMN1 gene (approximately 10% of cases). Furthermore, we used this method to improve the genetic counselling in couples formed by one SMA carrier and a person of the general population (1/200 potential risk of having an affected child). A total of 70 obligate carriers (more than one deleted affected child in the offspring) and 41 non-carriers (relatives of SMA families defined by marker studies) were analyzed to validate the method. DNA was amplified with specific fluorescent primers in a multiplex PCR including the Retinoblastoma (RB) gene (as genomic control), the SMN1 gene and their respective internal standards. Products were scanned in an ABI Prism 310 (Perkin Elmer). The sensitivity was 95.5% and the specificity 97.5%. Analysis of 29 SMA cases without homozygous deletion of the SMN1 gene, demonstrated single dose in 9 of them (31%). Further analysis of these cases revealed at least five cases of hybrid SMN1-SMN2 genes and a novel splicing mutation in intron 6. Furthermore, we have performed carrier studies to 25 different couples with an "a priori" risk of 1/200 and after a Bayesian calculation, we were able to reduce or increase their final risk significantly. The quantitative method for SMN gene dosage is a useful tool to apply in cases without homozygous deletion of SMN1 and in couples with 1/200 potential risk. In these couples it was possible to modify the final risk to less ambiguous figures to facilitate their decisions for prenatal diagnosis. The analysis can also be applied to families where the index case is death and no sample is available for study and to screen cases of semen donation of the general population. Supported by FIS 00-481 and Marato TV3.

COMPARISON OF MUTATION DETECTION METHODS FOR THE CONNEXIN 26 GENE. *S.D. Prasad, R.A. Cucci, R.J.H. Smith.* Otolaryngology, University of Iowa, Iowa City, IA 52242.

Mutations in GJB2 are the most common cause of hereditary congenital hearing loss in many countries and are found in about 50% of persons with severe-to-profound congenital autosomal recessive non-syndromic hearing loss (ARNSHL) (Zelante, Hum. Mol. Gen. 6:1605-1609,1997). GJB2 mutation screening provides much benefit, including the ability to diagnose the etiology of severe-to-profound congenital deafness and to provide parents and patients with improved genetic counseling data. The high prevalence of Cx26 mutations and their importance as a cause of ARNSHL have prompted the development of several different mutation detection assays to screen the single Cx26 coding exon. Since the entire coding exon has to be screened, comparative assays should be capable of mutation detection in the entire coding exon. Options include direct sequencing, single-strand confirmation polymorphism (SSCP) analysis, and WAVE HS Nucleic Acid Fragment Analysis (Transgenomic Inc). In this report, a battery of known Cx26 mutations was used to determine the optimum combination of techniques to provide rapid, inexpensive, and highly sensitive mutation detection. The limitations and benefits of these mutation detection assays are described in detail.

Mutations in the Notch3 gene in biopsy proven CADASIL patients. *C.A. Stolle¹, V. Kasparcova¹, J.J. Dowhanick-Morrisette², N.B. Spinner², J. Farmer³, D.A. Brooks³, D.R. Lynch⁴.* 1) Dept of Genetics; 2) Div. Human Genetics, Department of Pediatrics; 3) Div. Medical Genetics; 4) Dept of Neurology, University of Pennsylvania, Philadelphia, PA 19104.

CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukencephalopathy) is an autosomal dominant genetic disorder, that predisposes to stroke, migraines, confusion, memory loss, and seizures. The onset of symptoms usually occurs at 35 years of age, though it is the accumulation of small strokes that may lead to memory loss or dementia. CADASIL is due to mutations in Notch3, a 33 exon transmembrane receptor gene that is involved in early cell fate decisions during embryonic development. The majority of mutations occur in the extracellular domain of Notch3 which contains 33 EGF-like repeats. Most are missense mutations that result in a gain or loss of a cysteine residue. We have developed a multilevel clinical assay for mutations in the Notch3 gene in CADASIL patients. This assay involves mutation scanning by conformational sensitive gel electrophoresis followed by DNA sequence analysis of exons exhibiting a shift. Level I includes those exons (3, 4, 9, 11, 18, and 19) in which the majority of mutations have been identified. This represents 86% of identified mutations. Level II includes exons in which a single mutation has been reported (exons 2, 5, 12, 14, 20, 22, 23, and 25). Level III involves the remaining exons starting with exons encoding EGF-like repeats. Four patients with a family history of stroke and a skin biopsy indicative of CADASIL have been screened. Mutations were found in all four patients; two mutations were identified in exon 3 (level I) as well as one mutation each in exons 2 and 25 (level II). Two of these mutations involved loss of a cysteine and one involved gain of a cysteine. One mutation was a missense in the Notch/Lin12 domain. Three of these mutations had not been reported previously. CADASIL represents a late onset disease which may be under diagnosed in the stroke population. Our experience suggests that a rapid and cost effective clinical assay for CADASIL may be possible based on limited testing of mutation rich exons.

Molecular diagnoses of neuronal ceroid lipofuscinoses (NCLs). *D. Moroziewicz*^{1,2}, *W. Ju*^{1,2}, *A. Jurkewicz*^{1,2}, *K. Wisniewski*^{3,4}, *W.T. Brown*², *N. Zhong*^{1,2,4}. 1) Molecular Neurogenetic Diagnostic Laboratory; 2) Human Genetics, NYS Inst Basic Research, Staten Island, NY; 3) Dept. Neuropathology³, NYS Inst Basic Research, Staten Island, NY; 4) Dept. Neurology⁴, SUNY-Health Science Center at Brooklyn, Brooklyn, NY.

The neuronal ceroid lipofuscinoses (NCLs), or more commonly called Batten disease in lay population, are a large group of autosomal recessive lysosomal storage disorders with both enzymatic deficiency and structural protein dysfunction. Three typical forms, the infantile (INCL), late-infantile (LINCL), and juvenile (JNCL), are among the most common childhood-onset neurodegenerative disorders. They result from mutations on genes CLN1, CLN2, and CLN3, respectively. We determined that the mutations 223A->G and 451C->T in CLN1, IVS5-1 and 636 C->T in CLN2, and deletion of a 1.02-kb genomic fragment in CLN3 are the five common mutations for NCLs. In order to offer clinical genetic testing, we have developed simple and quick PCR-based molecular tests for detecting INCL-, LINCL-, and JNCL-affected individuals from 252 NCL families (34 INCL, 109 LINCL, 98 JNCL, and 11 unclassified). Sensitivity of testing these five common mutations is about 70% in all clinically referred NCL cases. Seven patients previously diagnosed as having JNCL were now found to carry mutations of CLN2 (5/7) or CLN1 (2/7) and three with late-infantile onsets were identified as carrying mutations of CLN1. In addition, several novel mutations have been identified. Applying our testing, carriers and pre-symptomatic individuals can be identified. Our data demonstrate the importance of DNA testing, which may provide a fundamental information for the enzyme replacement or gene therapy for the NCL families in the near future.

Community genetics and public health in Cuba. *L. Heredero, L. Rodriguez, J. Quintana, J.A. Oliva, A. Lantigua, R. Guel, B. Barrios, H. Granda.* National Ctr Medical Genetics, Havana Medical University, Havana, Cuba.

The current Cuban population is 11 million, annual number of births approximately 150,000 and infant mortality 7.0 per thousand. A program for the diagnosis and prevention of genetic disorders and birth defects has been in place in Cuba since 1982, under the oversight of the Maternal and Child Health Division of the Ministry of Health and under the direction of the National Center of Medical Genetics of the Medical University of Havana. As part of this program, the following services have been made available free of charge: clinical genetic diagnosis and counseling services (to all the Cuban population), routine prenatal detection of sickle cell carriers and of fetal malformations in the second trimester of pregnancy by maternal serum AFP determination and high resolution ultrasound at 20 weeks (to all pregnant women), prenatal diagnosis of chromosome anomalies (to all women over 38 y.o.), and newborn screening for PKU and congenital hypothyroidism (to all newborns). Molecular genetic testing is available for hemoglobin S, cystic fibrosis, fragile X and others, for pre- and postnatal diagnosis. Approximately 100 trained medical geneticists provide the above mentioned services. The genetic services program is directly linked with the primary care level through 500 family physicians nationwide trained in the detection of genetic risks and proper referral. Consequently, over 90 percent of the population benefits from these services, which has correlated with an improved quality of medical genetics care and a reduction in the infant mortality caused by birth defects. Currently, a program in public education in genetics is underway in order to have a more active involvement of the community in the genetic programs and to facilitate the future introduction of more complex approaches to genetic services such as presymptomatic testing and counseling for late onset mendelian conditions and for susceptibility testing and counseling for common medical conditions.

Melting curve Insertion/Deletion (McID): A low cost, high-throughput genotyping method for forensic identity analysis. *D.M. Sosnoski¹, E. Parra¹, J. Akey², M.D. Shriver¹*. 1) Dept. of Anthropology, Pennsylvania State University, University Park, PA; 2) University of Texas at Houston, Houston, TX.

It has been estimated that as many as 100,000 insertion/deletion polymorphisms of various lengths and base pair compositions are located throughout the human genome. Once characterized and assessed for allele frequency in the general population, these polymorphisms could be used as DNA markers for forensic identification or for mapping disease loci.

The traditional PCR gel based approaches for genotyping insertion/deletions do not allow for efficient high-throughput screening, while other polymorphism detection methods are both complicated and costly. We describe here a novel method, Melting curve Insertion/Deletion (McID), for genotyping insertion/deletion polymorphisms which takes advantage of the difference in melting temperature of two DNA fragments of unequal base pair composition.

Small segments of DNA (45-80 bp) which contain the polymorphism of interest are PCR amplified in the presence of SYBR Green and formamide. The products are then assayed by the Hybaid DASH system which heats the samples at a constant rate while measuring the fluorescence of the double stranded DNA. Sudden decreases in fluorescence denote the melting temperature of each DNA fragment and are plotted as negative first derivative peaks on the instrument readout. Typically, homozygous individuals are displayed as a single peak at a discrete temperature, while heterozygous individuals are observed as two peaks or one double peak. The DASH instrumentation allows for simultaneous assessment of 96 samples as well as computer automated scoring of the results; the McID assay can genotype and score 96 samples in less than 15 minutes at a cost of less than \$0.002 above the cost of a standard PCR reaction. Using a standard set of highly informative insertion deletion polymorphisms, McID provides a low cost automated method for large scale DNA fingerprinting.

Evaluation of a preventive program for Tay-Sachs disease in the Brazilian Ashkenazi Jewish population. *L. V. Pereira, R. Rozenberg.* Dept Biol, Inst Biociências, Universidade de São Paulo, São Paulo, SP, Brazil.

Tay-Sachs disease (TSD), an autosomal recessive disease of lysosomal storage characterized by progressive neurologic degeneration, causes death of affected children until the age of 5. TSD is caused by mutations in the HEXA gene, coding for the α subunit of the hexosaminidase A enzyme. In the Ashkenazi Jewish (AJ) population, the carrier frequency of TSD is 1 in 31 individuals. Three mutations account for 98% of the cases of TSD in this population, allowing the molecular diagnosis of affected children and carriers. Preventive programs have been available for Ashkenazi Jews and French-Canadians since the 70's in the USA, Canada and Israel. Their goal is to detect and provide genetic counseling for couples at risk of having affected children. Over 1,000,000 people have participated in these programs, leading to a 90% decrease in the incidence of TSD. In Brazil, the Jewish population is of approximately 100,000 individuals. We evaluated the need and the acceptance of a preventive program for TSD in the Brazilian Jewish population. Educational classes about TSD were presented to Senior students of Jewish high schools. After the presentation, students took home a consent form with a brief explanation of the program and a questionnaire to the parents. With the parents agreement, DNA was extracted from mouth mucous of the participants. Presence of the 3 most common AJ TSD mutations was analyzed (1278insTATC, IVS12+1, Gly269Ser). Results and their interpretation were confidentially sent by mail to the students. Among 581 students, 449 chose to get tested (77% participation rate). 98% of the parents rated the program "important" to "essential". Eight carriers were found among 326 students tested, indicating a carrier frequency in the Jewish schools of 1 in 41. Taking into account only the chromosomes of AJ ancestry, we estimate a carrier frequency of 1 in 30, equivalent to that observed in the AJ population in general. These results corroborate the implementation of a TSD preventive program in the Brazilian AJ population until a cure or treatment for the disease is developed. FUNDING: FAPESP.

Detection of bacterial strains by real-time PCR using the LightCycler™. *F. Basalyga, A. Davis, B.H. Thompson, J.T. Simpson.* Center for Medical and Molecular Genetics, Armed Forces Institute of Pathology, Rockville, MD.

The clinical effects of Group B *Streptococcus* (GBS) and Chlamydia infections are very serious. It is estimated that 10-30% of pregnant women are colonized with GBS and vertical transmission, from mother to neonate, may lead to infection of the infant. Several factors have been associated with early onset GBS disease in the newborn, including premature deliveries, prolonged rupture of membranes or intrapartum fever. Current mortality rates for these infected newborns are 5-20%. Chlamydia (*C. trachomatis*) is the most prevalent sexually transmitted disease in the US. It can cause infections of the cervix, urethra and upper genital tract in women, infections of the urethra and epididymis in men, and conjunctivitis in newborns. *C. pneumoniae* has been linked to atherosclerosis and asthma. We have an ongoing interest in the rapid analysis and identification of clinical virulent bacterial strains. In this study, we present recent results in the identification of *C. trachomatis*, *C. pneumoniae*, and Group B *Streptococcus* using LightCycler™ technology.

Bacterial strains were identified using a LightCycler™ thermocycler (Roche Molecular Biochemicals). The LightCycler™ is optimized for rapid PCR applications and complex applications such as product analysis, quantitation, and mutation analysis can be completed in approximately 20-30 min. The technique is based on real-time fluorometric analysis of the PCR products produced. Comparison of melting temperatures can be used to identify bacteria and differentiate between strains. A SYBR Green I kit has been used to differentiate *C. trachomatis* from *C. pneumoniae* and Group B *Streptococcus*. GBS can be further identified from cell suspension and clinical samples using primer sets specific for amplification of one of nine large tandem repeating units present in the C protein alpha antigen gene (bca).

Brief breast cancer risk assessment at the time of breast biopsy: triage and referral. *S.M. O'Neill^{1,2,3}, V.G. Vogel², E. Feingold¹, J.A. Peters^{1,3}, R.E. Ferrell¹, W.S. Rubinstein^{1,2,3}.* 1) Dept. Human Genetics, Univ. Pittsburgh, PA; 2) Comprehensive Breast Program, Univ. Pittsburgh Cancer Institute/Magee-Womens Hospital, Pittsburgh, PA; 3) Cancer Genetics Program, UPCI/MWH, Pittsburgh, PA.

Referral of women at high risk of breast cancer to cancer risk counseling (CRC) services is unstandardized in general clinical populations, and little is known about factors that influence women to follow-up with referral. A brief computerized quantitative breast cancer risk assessment (BRA) may be a useful method for identification of referral candidates, but information is needed about patient attitudes and interest in genetic counseling. We studied 100 women attending a comprehensive breast program for fine needle, core, and stereotactic breast biopsies. Subjects had BRA using the Gail and Claus models; questionnaires measuring knowledge and attitudes about breast cancer, CRC, and genetic testing; and psychological assessments using the Profile of Mood States (POMS) and the Impact of Events Scale (IES). Those with calculated risk ≥ 2 x the age-related population risk were referred to the Cancer Genetics Program for comprehensive risk assessment and counseling. The study population was of high socioeconomic status (96% had health insurance) and 37% reported a 1st or 2nd degree affected relative. CRC referrals were provided to 53 women, with Gail 10-year risks ranging from 1%-17%. Within 6 months of referral, 1 subject came for consultation. The remaining referees were contacted 3-6 months post-biopsy. Reasons cited for not following up included: enough information provided by the BRA (40%) or their physicians (49%), low perceived risk (31%), no family history (31%), and negative biopsy result (17%). However, 46% stated that they would consider genetic counseling in the future and cited precipitating events such as another biopsy, a cancer diagnosis, or a relative's request. Objective risk may not be a sufficient motivation for seeking CRC for women assessed at the time of breast biopsy. Intention to follow up may depend on factors relating to immediacy of threat. Subjects diagnosed with breast cancer cited treatment priorities and dissuasion from their physicians as reasons for postponing CRC.

Public Understanding of Genes and Genetics: Implications for the Utilization of Genetic Services and Technology. *E.M. Petty, S.R. Kardia, R. Mahalingham, C.A. Pfeiffer, S.L. Saksewski, M.G. Brandt, E.S. Anderson, T.E. Jayaratne.* University of Michigan, 4301 MSRB III, Ann Arbor, MI 48109-0638.

The explosion of genetic information and technology over the past decade has undeniably impacted many areas in society including medicine. Acquisition of further genetic knowledge and related technology during this next decade will have significant far reaching implications for virtually all members of society, especially as related to health care. Given this, stories about genes and genetics - genetic tests, genetic predispositions, genetic diseases, and genetic manipulation - have been widely publicized in the media. What is conveyed, however, when we say something is genetic? While a definition among ASHG members may differ to some extent, a wide range of diverse responses would be anticipated from individuals lacking scientific expertise in this area. As part of an ongoing project exploring genetic beliefs for individual and group differences, we needed to understand how respondents understood the concepts of genetics and genes. During in depth one-on-one structured telephone interviews with adults in the US, we specifically asked questions exploring their basic understanding of genetic concepts. When asked what was meant if something was 'genetic' many referred to it as something with a familial basis, obtained at birth from parents or ancestors. Others noted it was simply 'ingrained in us' or our 'instincts'. Few mentioned any connection to genes, DNA, and/or chromosomes. Some even defined genetics in more magical terms such as being related to 'brain waves' or 'in the stars'. When asked where genes were located in the body responses varied from 'in your whole fiber' to only specific organs or tissues such as the 'brain' or 'blood'. Few respondents demonstrated an accurate working understanding of general genetic concepts. Many noted frustration or were very hesitant when trying to define genetic concepts. This lack of understanding, along with misconceptions, about basic genetic concepts has significant implications for broader public education measures in genetic literacy, genetic counseling, and public health practices.

National Genetic Blood Disorders Project(student screening). *S.S. Al Arrayed¹, S. Ameen², N. Hafadh², H. Al Mukhareq³, H. Sanad⁴, F. Ali¹.* 1) Genetic Department, Salmaniya Medical Ctr, Manama, Bahrain; 2) MCH,.MOH; 3) Ped dept,MOH; 4) Pathology lab.MOH.

The inherited disorders of Hemoglobin including Thalassemia,sickle cell disease and Glucose Phosphate Dehydrogenase are common in Bahrain.The aim of the study was to update the national data,to raise awareness among youth and public about measures for prevention.- Material and method:The target group was all-Bahraini and non Bahraini students in 11th grade in all schools in Bahrain,nearly 7000 students.The project took 10 months for planning,education,collection of blood laboratory testing,data processing,and card distributio to students,data analysis and reporting.Parents permission was taken prior to testing,and the students were fully informed through educational sessions.HPLC machine was used for electrophoresis,testing for blood group and G6PD deficiency were also performed.Cards with the results were given to all students,as well as each school-received report on the prevalence of blood diseases.-Results:Total number of student screened was 5983,in 38 schools.Parents response rate was 81%. One hundred twenty educational lectures were given,and 30,000 educational booklets were distributed. We found that the prevalence of SCD is 1.2%.It is declining gradually as the previous figure was 2.1%- Sickle cell trait (SCT) 13% previous figure was 11%- Beta thal trait 2.9% and G6PD 25%.This study shows that there is variation among regions in Bahrain,Western area has the highest rate for SCT 25%,but it has lowest rate for beta thal 2%.Al Hidd region has the highest prevalence for Beta thal 5.4%.but the lowest for SCT 2.7%.Sitra region has the 2nd highest rate for SCT 21%,and 2nd highest for beta thal 5.3%. we report on the level of HB S,F,A2,and H in each group.Prevalence of abnormal hemoglobin such as HBD 0.56%,HBE 0.02%.Prevalence of Blood group and RH group are as follow.O RH+47%,B+ 23%,A+20%,AB+ 0.25%-Conclusion:Weupdates the national figure for the prevalence of genetic blood disease in Bahrain.It raises awareness in public especially among youth about such diseases.Each student received a plastic covered card with the results, which can be used for life.

Documentation of the cancer family history. *O.K. Gordon, L.S-C Cheng, M.T. Scheuner.* Cedars-Sinai Medical Center, Los Angeles, CA.

Family history is crucial for risk assessment and diagnosis of hereditary cancer syndromes. The purpose of this study was to characterize documentation of cancer family histories. Family histories were reviewed for newly diagnosed cases of breast, ovarian, colon, prostate, and lung cancer seen in 1998 at Cedars-Sinai. The family history was scored as not recorded, non-contributory, none, positive, or other. Variables considered were Jewish ancestry, gender and age at cancer diagnosis. 1,459 cases were reviewed including breast, 532; prostate, 308; lung, 283; colon, 253; and ovarian, 83. 61% were female. 34% were Jewish. Family history was not recorded for 22% of cases including prostate, 32%; colorectal, 26%; lung, 22%; ovarian, 17%; and breast, 15%. Family history was non-contributory in 7%. Approximately one third of cases had a positive family history and another third had none. Breast and ovarian cancer cases were most often positive, 43% and 42%, respectively, compared to 30%, 33% and 34% for prostate, lung and colorectal cancer. Thus there were more positive family histories for females. 41% of females had a positive family history compared to 29% of males, $p < 0.001$. Correspondingly, 37% of males had no recorded family history or non-contributory histories compared to 23% for females, $p < 0.001$. Positive family histories were documented more often for cases diagnosed before age 70 compared to those diagnosed at age 70 or later, 44% vs. 30%, $p < 0.001$. Documentation of family history was similar for Jewish and non-Jewish cases. In conclusion, 22% of newly diagnosed cancer cases lacked any documentation of family history, and an additional 7% were classified as non-contributory. Older patients and males more often lacked documentation of family history suggesting lack of appreciation of family history in determining a genetic susceptibility, which may result in under-utilization of genetic services and appropriate preventive strategies for genetically susceptible patients and their family members. Implementation of a tool for systematic family history collection may improve these statistics. These results underscore the need for physician education regarding the value of genetics for their cancer patients and their relatives.

Recognition of high risk individuals for hereditary cancer syndromes and referral by comprehensive cancer center staff. *K. Sweet, T. Bradley, J. Westman.* Human Cancer Genetics Program, The Ohio State University, Columbus, OH.

Identification of families demonstrating hereditary cancer is necessary for comprehensive cancer management and provision of genetic consultation and testing. Oncology staff at a Comprehensive Cancer Center have historically used family history questionnaires to determine individual risk and appropriateness for genetic counseling referral. The clinical cancer genetics staff developed a touch-screen family history computer program (JamesLink) which allows a patient to enter family cancer history information for risk assessment. A chart review was performed of 396 patients who had voluntarily entered family history information into JamesLink. The medical record information versus the JamesLink entry was compared for accuracy of family history information and risk assessment. The number of referrals for genetic consultation was determined and compared to the level of risk assessment assigned by the clinical cancer genetics staff. Of the 396 JamesLink entries, 50 (12.6%) were classified at high risk for a hereditary cancer syndrome. Surgical oncology staff had seen 70% of these individuals; 22% were medical oncology patients. Of these 50 high risk patients, only 2 (4%) had a notation in their record for being at high risk and were referred for genetic consultation. Only 7 (14%) of the high risk records contained adequate family history information for assessment. Notable indicators of hereditary cancer syndromes (age at diagnosis, ethnicity, history of second degree relatives) were generally not addressed in the chart. Family history of a single index cancer was taken while other relevant cancer history was infrequently documented. Individuals at high risk for hereditary cancer syndromes may not be appropriately referred even in the comprehensive cancer setting. Design and use of family history questionnaires without the input of cancer genetic professionals were inadequate for risk assessment. Even when appropriate information was obtained it was not assessed properly by either medical or surgical oncology staff. Targeted educational interventions and clear cancer genetic counseling referral criteria are needed to improve risk assessment and patient care.

A pilot program for the prevention of genetic diseases in inbred communities. *J. Zlotogora*^{1,2}, *S. Shalev*², *A. Leventhal*³. 1) Community Genetics, Ministry of Health, Jerusalem, Israel; 2) Genetic Unit, Hahemek Medical Center, Afula, Israel; 3) Public Health Services, Ministry of Health, Jerusalem, Israel.

Genetic disorders are relatively frequent among Arabs who represent some 20% of the Israeli population. Among the autosomal recessive diseases, some like thalassemia, deafness and FMF are frequent in the whole population. However, for most diseases the high frequency is limited to a small region, a single village or an expended family. Therefore, the distribution of genetic disorders is different from one village to the other. In addition, the marriages are not only by preference within the family (more than 22% first cousin marriages and 45% consanguineous) but also within the village or a small geographic region. Taking in account these observations we begun a pilot program for the prevention of genetic disorders in two different villages. The program includes:

- (1) Detailed analysis of the the genetic diseases present in each of the villages and determination of their molecular basis.
- (2) Education program on the medical effect of consanguinity elaborated and implanted in cooperation with the local secular, political and religious leaders.
- (3) Genetic counselling given locally, within the community.

In the first village (8,600 inhabitants), up to now 20 different autosomal recessive diseases have been diagnosed. Among those 4, have been diagnosed in more than 2 families (deafness, retinitis pigmentosa sickle cell anemia/thalassemia and a renal disorder). In the second village (5,000 inhabitants), among the 14 autosomal recessive diseases diagnosed, 4 were present in more than 2 families (cystic fibrosis, congenital nephrotic syndrome and two previously undescribed syndromes).

The program has been well accepted within each community. It already allowed us to offer more accurate genetic counselling including in some cases screening tests for carrier detection and/or prenatal diagnosis.

Methylation-sensitive PCR (MS-PCR) analysis of the fragile X (FRAXA) syndrome. A. Weinhaeusel, C. Skarits, O.A. Haas. Children's Cancer Research Institute (CCRI) and Ludwig Boltzmann-Institute for Cytogenetic Diagnosis (LBICD), St. Anna Children's Hospital, Vienna, Austria.

The FRAXA syndrome is the most common cause of inherited mental retardation in males. Its perplexing molecular genetic pathomechanism and its unusual pattern of inheritance pose an extraordinary challenge for its diagnostic evaluation in the laboratory. We have therefore developed a MS-PCR-based test-system that takes advantage of the varying repeat lengths that define normal, premutation and full mutation *FMR1* alleles. In addition, it accounts for the different methylation patterns of the CGG repeat and the promoters of the *FMR1* and *XIST* genes that are encountered in nonaffected and different types of affected males and females. The *XIST* gene promoter, whose allelic methylation pattern opposes that of the *FMR1* promoter, serves as an internal control and standard for semiquantitative analyses. Deamination of the DNA with sodium bisulfite prior to amplification converts all unmethylated, but not methylated, cytosine residues to uracil, which is replaced by thymidine during subsequent DNA replication. This procedure generates derivative sequences from originally homologous, but differentially methylated sequences and by that also significantly improves the PCR conditions for both unmethylated and methylated DNA. We evaluated our system in a blinded fashion on well-defined samples with various extraordinary forms of FRAXA mutations. Furthermore, we used it as a routine screening tool for the diagnostic evaluation of so far 253 male and 80 female probands that were referred to our laboratory. In the male group, we identified five full mutations, and three premutation and gray zone alleles with 54, 55 and 62 repeats, respectively. The female group consisted of 33 normal homozygote and 41 heterozygote individuals, two of whom harbored a gray zone allele with 47 repeats, none with a premutation and six with a full mutation. In addition, we have analyzed 81 samples from individuals with constitutional and acquired sex chromosome abnormalities (Turner, Klinefelter, multiple X syndromes and leukemia patients) in order to study clonality and skewing phenomena.

Construction of a 10,000 element human eye cDNA array for distribution to the scientific community. E.A.

Donorum^{1,3}, J. Graff^{1,2}, K. Brown^{1,2}, D. Smith³, A. Behal³, K. Peterson³, S. Bernstein⁵, D.A. Stephan^{1,4}, G. Wistow³. 1) Research Center for Genetic Medicine, CNMC, Washington, DC; 2) George Washington University School of Medicine, Washington DC; 3) National Eye Institute, NIH, Bethesda, MD; 4) National Human Genome Research Institute, NIH, Bethesda, MD; 5) University of Maryland, Baltimore, MD.

A 10,000 element cDNA array is being constructed to serve the array needs of the intra- and extramural scientific community. Expression profiling in various eye disorders would be an invaluable reagent in the search to identify etiologic and therapeutic targets. We are currently constructing such an array for use as a standard reagent, data from which will be databased and web accessible, available on a collaborative basis. ~20,000 clones have been sequenced from normalized and non-normalized 3' cDNA libraries constructed from lens, iris, RPE/choroid, fovea, cornea and retina. These sequences have been aligned into ~10,000 unique EST/gene clusters. Representative ESTs of ~1-2kb insert length, from each cluster, have been chosen and picked into 96-well format. These ~100 plates will be amplified and the amplicons printed onto CMT-GAPs glass slides in batches of 100 arrays. Approximately 1,000 arrays are planned. Concurrently, we are working with collaborators to reduce the required total RNA sample size needed for template preparation into the range which is accessible from non-pooled human clinical samples (1ug range). All data from array collaborators will be posted to our web site and images and raw extracted intensities as well as calibrated ratios can be downloaded (<http://www.childrens-research.org/microarray/microarray.html>). It is our hope that such a reagent coupled with standardized sample and data processing will aid in establishing the gene expression anatomy of the human eye.

Double-stranded RNA binding protein nuclear factor 90 (NF90) is involved in transcriptional activation of antiviral pathway genes causing resistance to HIV-1 *in vitro*. I. Riz¹, A. Spruill¹, A. Kumar¹, D.A. Stephan^{2,3}. 1) Biochemistry and Molecular Genetics, George Washington University, Washington, DC; 2) Research Center for Genetic Medicine, CNMC, Washington, DC; 3) NHGRI/NIH, Bethesda, MD.

A small subset of HIV infected individuals do not develop AIDS. Expression array analysis of an *in vitro* model of resistance to HIV-1 infection yielded potential insights into such protective mechanisms. We recently cloned a DRBF gene that is a member of a family of double stranded RNA binding proteins with a variety of functions (NF90). Among this family are TCP-80 (which inhibits protein translation of several genes) and MPP4 90 and 110 (which have M phase specific phosphorylation). In this study we found that cells expressing NF90 are resistant to HIV-1 infection/replication. Moreover, transient transfection of NF90 into a portion of cells in culture prior to infection is enough to protect the whole cell culture population against infection, suggesting intracellular signaling mechanisms. We used our NF90 GHOST cell transfection system (genetically homogeneous except for the NF90 protein) to identify genes that are dysregulated and lead to protection against infection. Expression analysis was performed to examine the global changes in gene expression induced by NF90 with or without infection, as compared to untransfected controls. Among ~5600 full-length genes on the HuGeneFL Affymetrix array and ~6,500 genes and ESTs on a custom cDNA array which were tested, we found that 94 genes were differentially regulated four fold or more in cells constitutively expressing NF90. Transcripts from promoters containing interferon responsive elements (ISRE) comprised the most distinctive group of similarly regulated gene products. Interferons and ISRE-containing genes have been implicated in cellular defense mechanisms in response to RNA virus replication. Can NF90 directly interact with these promoters or it is activating components of JAK-STAT pathway? Protein complexes formed on these promoters are under investigation in order to elucidate the molecular mechanism of resistance to HIV-1 infection/replication.

Homophila: A cross-genomic relational database of human disease genes to the complete *Drosophila*

***melanogaster* genome.** E. Bier¹, L. Reiter¹, L. Potocki², M. Gribskov³. 1) Department of Biology, UCSD, La Jolla, CA; 2) Baylor College of Medicine, Houston, TX; 3) Super Computing Center, UCSD, La Jolla, CA.

With the completion of the *Drosophila melanogaster* genome sequence a new age of genomics has begun. In addition to the clear implications of knowing all the genes required to create this complex organism, the time is now ripe for applying our detailed knowledge of *Drosophila* biology to the analysis of human disease causing genes. Currently available GAL4-UAS technology allows us to mis-express human genes in a variety of expanding temporal, spatial and developmental specific patterns. Our group has been developing a database which will aid in the rapid identification of human disease gene homologues present in the *Drosophila* genome which would be good candidates for deciphering the etiology of human disease. Although a preliminary survey of a representative group of human disease homologues was presented at the time of the genome sequence release (Rubin et. al. 2000. *Science*, 287: 2204-15) we present here a web based comprehensive BLAST analysis of all human disease genes present in the Online Mendelian Inheritance in Man (OMIM) compared to the complete genome sequence of *Drosophila* in the form of an updateable and searchable database. We plan to expand this database to include phenotypic information from both the human and *Drosophila* sides of the genetics community. Our initial analysis of the set of human disease homologues indicates that there are 682 genes which match a given entry in the OMIM disease gene list at a P_{value} better than e^{-20} . Of these genes, 312 appear to be in known *Drosophila* genes and the remainder in genes recently discovered during the completion of the genome sequence. Preliminary analysis indicates that there are several good candidate genes for the study of eye disease, deafness, heart defects, muscular dystrophies, neuropathies and cancer in *Drosophila*. We anticipate that the construction of this dynamic database (<http://homophila.sdsc.edu/>) will encourage cross communication between researchers in human and *Drosophila* genetics and will encourage wide use of *Drosophila* as a model system for analysis of human disease phenotypes.

Statistical methods for gene expression studies using serial analysis of gene expression (SAGE). *W.F. Forrest, D.G. Peters, E. Feingold.* Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

Serial Analysis of Gene Expression (SAGE) is a powerful technique that produces a quantitative global analysis of gene expression. Its chief advantage over microarray methods is that it does not require prior knowledge of the genes of interest. SAGE produces an exact count of the number of each species of mRNA seen in a subsample. SAGE is labor-intensive, and is generally only done on a small number of samples, often as a "first-pass" to be followed up by microarray studies. Statistical methods for analysis of SAGE data can be similar to those used for microarray data, except that they must take into account the small sample size and the unique multinomial sampling structure produced by counting mRNAs in a subsample. We present a log-linear model for SAGE data that captures both the multinomial sampling structure and sample-to-sample variability. We show how to use our model to analyze SAGE data, with particular attention to the problem of producing a "short-list" of genes for further study. We demonstrate our methods on publicly-available datasets.

Searching for the RP10 gene: a model for positional candidate cloning in the post genome era. *S.J. Bowne¹, D.L. Tirpak¹, L.S. Sullivan¹, A. Kennan², P. Humphries², S.P. Daiger¹.* 1) Human Genetic Ctr. and Dept. of Ophthalmology, Univ. of Texas-Houston; 2) Genetic Dept., Trinity College, Dublin, Ireland.

Identification of disease-causing genes using positional candidate cloning is an important part of genome research as sequencing of the human genome nears completion. The RP10 locus, which maps to a 5cM region between markers D7S686 and D7S530 on 7q31 and is a major cause of autosomal dominant retinitis pigmentosa, is a model system for applying post-genomic positional cloning procedures. Contiguous assembled genomic sequence spans 65% of the RP10 region, while approximately 30% is covered by unassembled shotgun sequences which contain several small gaps. By using the genomic sequence from the non-redundant (nr) and high throughput genomic sequence (htgs) databases at NCBI, we designed several new polymorphic markers for fine-point haplotype analysis. This analysis has led to a shifted and reduced region, 4cM in size. Identification of candidate genes located in the minimal critical region has been slow with GeneMap 99 only increasing the number of genes in this region to 48. We have designed a protocol for using the emerging genomic sequence to localize candidates to the RP10 region. First, an STS map of 7q31 is used to identify and orient large genomic sequences from the region as they are generated and deposited in GenBank. These sequences are broken into 50,000 bp segments, repeat masked, and searched by BLAST analysis for known genes or ESTs. ESTs mapping to the genomic sequence are assembled into clusters using TIGR, UniGene, and manually. With this procedure we analyzed 70% of the RP10 critical region and identified over 130 potential genes, and determined that 8 of the genes identified by GeneMap 99 are not contained in the critical region. The remaining 30% of the existing sequence, and any new sequence generated for this region, will be analyzed using the same procedure. Genes identified in this search will be prioritized as candidates based on retinal expression. This approach has a high likelihood of identifying promising candidate genes for RP10 and can be applied to successfully identify other disease-causing genes.

Disease-gene discovery pipeline. *T.A. Braun, T.L. Casavant, V.C. Sheffield.* Departments of Genetics, Electrical and Computer Engineering, and Pediatrics, The University of Iowa, Iowa City, IA.

The Human Genome Project (HGP) has generated sequence and maps used by investigators to aid in their efforts to find disease-genes. The large amount of information currently available makes the utilization of all available information and resources impractical without the use of automated software applications. This trend of increasing amounts of information will continue as the completed sequence will make possible the creation of high resolution maps, and similar sequencing and mapping projects in model organisms will enable the construction of comparative maps across organisms. We are developing applications to take advantage of and utilize the overwhelming amount of information available for use in identifying human disease-genes. This disease-gene discovery pipeline is a collection of applications and resources designed to automate the underlying processing of this large amount of data associated with locating and prioritizing candidate disease-genes. The disease-gene discovery pipeline is used to obtain and prioritize lists of known and mapped genes and periodically scan sequence databases and maps to determine the availability of sequences and gene information for particular intervals. The system uses custom applications to organize and manage sequence within a region of interest for further analysis. The central controlling application is a Java-based program that directs map-based searches, acquires and manages sequence, and distributes additional organizing processes and analyses. The custom applications include a novel clustering program that enables efficient use of the highly redundant available sequence by selecting representative elements to reduce sequence overlap, and a distributed blast server that schedules processes based on computational load and machine availability. Currently the system is being used to track multiple regions of interest for numerous disorders including autism and Bardet-Biedl syndrome.

Discovery Manager™: A Database and Bioinformatics Tools Useful for Investigations of Complex Disease. *S.M. Colby, T.G. Marr.* Genomica Corporation, Boulder, CO.

Discovery Manager™ software (Genomica Corporation) is designed to provide researchers with sophisticated computational tools to accelerate investigations of complex disease using current approaches involving gene finding, association analysis, or functional analysis. Researchers can gather and merge genetic, epidemiological, molecular, and biochemical data from their own research, the scientific literature, public databases, and Genomica's Reference Database. Such data is represented in a simple, intuitive, and reproducible manner within a database whose contents can be securely queried and navigated. Without being an expert in bioinformatics, researchers can use Discovery Manager's tools to store, display, query, analyze, and manipulate the data. Genetic or phenotypic data can be exported quickly to third-party programs like CRIMAP, MULTIMAP, GENEHUNTER 2.0, LINKAGE, MAPMAKER/SIBS, SAGE/SIBPAL, DISEQ, HAPLO, SIMWALK 2, GAS, and SAS. In many cases, results can be re-imported for display and further analysis. In addition, researchers own analysis programs can be invoked by Discovery Manager through a command line interface. Genetic, radiation hybrid, cytogenetic, and contig maps can be represented, compared, and queried using a standard set of tools. Computational tools for sequence analysis have been integrated and include BLAST 2.0, Chang-Marr, ClustalW, FASTA, msa2.1, and MZEF. ApoCom Grail™ and GenScan™ or researcher-specific algorithms can also be launched from within Discovery Manager. Furthermore, multiple sequence analysis experiments can be configured and automated in a conditional pipeline. Application of Discovery Manager to a genome-wide linkage scan and an association study with candidate genes illustrates how the tools can be combined flexibly to support different workflows.

Gene Expression Changes of Human Neutrophils After Exposure to Bacteria. *Y.D. Beazer-Barclay¹, S. Yamaga², H.J. Lee², S.M. Weissman², U. Scherf¹, J. Vockley¹.* 1) Gene Logic Inc., Gaithersburg, MD; 2) Department of Genetics, Boyer Center of Molecular Medicine, Yale University School of Medicine, New Haven, CT.

Neutrophils are the first cells to be recruited from the blood stream to sites of infection. They are critically important for determining the outcome of acute infections. Mature neutrophils are post-mitotic cells that synthesize lower levels of protein and RNA than do dividing cells. Nevertheless, they are actively phagocytic, and also contain a number of preformed anti-bacterial proteins including bactericidal peptides and a system for generating reactive oxygen species. Using an in vitro model of bacterial infection, we have analyzed the expression pattern of human neutrophils following exposure to bacteria. Neutrophils extracted from human blood were exposed to *Escherichia coli* for thirty minutes and two hours. The cell biology of the activation of neutrophils has previously been studied, but these studies have been limited to a few cytokines. The advent of the microarray technology now allows a global survey of changes in gene expression. We used high-density DNA oligonucleotide microarrays to measure gene expression changes of 12,000 full-length genes with approximately 7,000 known genes. Several hundred genes showed greater than 3-fold expression change including known cytokines and genes involved in neutrophilic stimulation, as well as genes previously not associated with neutrophil activation.

Bioinformatics of genome-wide experimental approaches: Design and development of correlative "GeneLists" and disease-specific Affymetrix chips. *R.H.A. Borup¹, Y.W. Chen¹, S. Toppo², G. Lanfranchi², G. Valle², E.P. Hoffman¹.* 1) Center for Genetic Medicine, Children's Research Institute Washington , DC; 2) CRIBI Biotechnology Centre University of Padua, Padua, Italy.

Genome-wide expression profiling of patient tissues using Affymetrix or cDNA microarrays is widely believed to hold tremendous promise for understanding disease etiology, pathophysiology, and monitoring of therapeutics. cDNA microarrays are less expensive than Affymetrix chips to produce, and are predicted to have good sensitivity, but there are major concerns regarding specificity of hybridization signals; and it can be difficult to compare data between experiments due to issues of quantification and choice of internal control RNA. Affymetrix expression profiling is a highly redundant and informatics-intensive approach, with 40 queries per gene, which improves consistency and cross-experiment comparison, however this platform is more expensive. The choice of platform and the specific set of genes to test are critical aspects of experimental design.

Here, we describe the rationale and methods for production of a tissue-specific Affymetrix MuscleChip. Our MuscleChip combines 2,100 genes from a non-normalized EST sequencing project resource (University of Padova), with 1,150 diff calls from experiments in Duchenne dystrophy, and alpha-sarcoglycan-deficient human muscle biopsies using Affymetrix HuFL stock chips. This approach provides complete ascertainment of genes expressed at reasonably high levels in normal muscle, together with genes important in pathophysiology of muscle disease which may or may not be muscle specific.

To define the sensitivity and specificity of the two experimental platforms, we have developed methods of cross-referencing data, and developing disease-specificity and platform accuracy indices for each gene under study. These correlative databases, we have termed GeneLists, cross-reference to accession number and provide statistical analysis of consistency of expression of genes between experiments, and across experimental platforms. Application to the inherited muscular dystrophies is shown.

A simple, efficient procedure for mapping SNPs to genomic sequence. *P.S. Chines¹, K. Silander¹, N. Narisu¹, M.R. Erdos¹, A.K. Voltz², K. Mohlke¹, F.S. Collins¹.* 1) GMBB, NHGRI, Bethesda, MD; 2) IDRB, NHGRI, Baltimore, MD.

Finding allelic association using a dense map of SNPs appears to be one of the most promising approaches in mapping complex disease genes. As of June 5, over 135,000 SNPs and 87.8% of the human genomic sequence is available in draft or finished form in NCBI databases. But applying these resources to generate dense SNP maps is not yet straightforward. We present a simple, fast method for finding SNPs that map to a region of interest. **Method:** We downloaded draft and finished sequence in a region of interest in FASTA format. We also downloaded SNP sequences and formatted them for e-PCR, including information on SNP alleles and position within PCR product. Using a version of e-PCR modified to report which strand was hit, we mapped the SNPs to the FASTA sequence. A Perl script calculated the exact position of the SNP within each clone and extracted flanking sequence. From this list, we selected SNPs based on location, available allele frequency and confirmation status. Finally, we BLASTed the flanking sequences of selected SNPs against NCBI's HTGS database to identify repetitive and non-specific sequences. **Result:** We mapped 686 SNPs to a 16cM region of Chromosome 20q, using the sequence of 83 clones available in draft or finished form (of 99 in the minimal tiling path), while NCBI's website showed 1539 refSNPs mapped to all of Chromosome 20, with 322 SNPs identifiable in our region.

Using e-PCR for initial screening, while reserving BLAST for evaluating the uniqueness of a limited set of selected SNPs, makes this method both faster and easier than using BLAST alone. Because SNPs are mapped to precise clone coordinates, duplicates are easily identified, and the SNP sequence is confirmed by comparison with high-quality genomic sequence. Arbitrarily long stretches of genomic sequence can be extracted for designing SNP genotype assays. The approach described here allows researchers to produce a dense SNP map for a given region of interest based on the most up-to-date resources available to supplement the pre-computed data from NCBI. The software used in this effort is available at <http://genome.nhgri.nih.gov/mapsnp>.

Mouse Genome Informatics: Alleles and Phenotypes. *T. Chu, C.M. Lutz, J.A. Blake, J.E. Richardson, J.A. Kadin, M.T. Davisson, J.T. Eppig.* The Jackson Laboratory, Bar Harbor, ME.

The Mouse Genome Informatics (MGI) website provides integrated access to data on the genetics, (comparative) genomics, (comparative) mapping, gene expression, strains and tumor biology of the laboratory mouse. MGI can be accessed at <http://www.informatics.jax.org>.

Because of the overwhelming resemblance of its genetic makeup to that of humans, the laboratory mouse has been exploited as the premium mammalian model for human genetic diseases. The Mouse Genome Database (MGD), one of the projects of MGI at The Jackson Laboratory, has expanded its representation of phenotypes and alleles to provide a new "Allele Query Interface" to provide information about mutant mouse genes in an organized yet flexible manner.

The Allele webpage of MGD has sought to organize mouse allelic information so that it is more accessible to users. Users can construct complex queries against allelic data that consider gene symbol/name, allele symbol/name, allele types (how an allele was created), inheritance modes (how allelic combinations affect phenotype), and molecular mutation (describe the physical change in a DNA molecule). This newly expanded phenotype and allele representation contains short gene reports and provides text string searches against MGI and the Online Mendelian Inheritance in Man (OMIM). It is our goal to adopt a fuller representation of mouse phenotype information so as to enhance a higher level of data integration and to provide controlled vocabularies for disease models for more flexible querying.

MGD continues to annotate for the scientific community comparative mammalian genomic information and extensive data on the mapping of mouse genes. Combined with the detailed gene reports and expanded allele representations, MGD is a highly integrated model organism resource.

MGD is supported by NIH grant HG00330.

Identification of genes that are differentially expressed in extraocular and limb muscle. *C.U Neimann, T.O Krag, T.S Khurana.* Clinical Biochemistry, Glostrup Hospital, Glostrup, Denmark.

The extraocular muscles (EOM) are anatomically and physiologically distinct from other striated muscles in mammals. Among other differences, they can be driven to generate individual twitch contractions at an extremely high frequency and are resistant to $[Ca^{2+}]$ induced myonecrosis. While EOM are preferentially targeted in some neuromuscular diseases such as myasthenia gravis and congenital fibrosis of the extraocular muscles, they are enigmatically spared in Duchenne's muscular dystrophy, despite the widespread damage seen in all other skeletal muscle groups during the course of this disease. To address the molecular mechanisms that specify the EOM-phenotype, we characterized the transcriptional profile of genes expressed in rat EOM versus limb muscle using a differential display strategy. Ninety-five putative differentially expressed cDNA tags were cloned, from which fourteen were confirmed as being differentially expressed by RNA slot blot and northern blot analysis. Ten of these cDNAs were homologous to known human or murine genes and ESTs, while four genes that were upregulated in EOM were novel, and have been designated Expressed in Ocular Muscle (eom) 1-4. The identification of these differentially expressed genes may provide mechanistic clues toward understanding the unique patho-physiological phenotype of EOM.

The Skeletal Genome Anatomy Project (SGAP): A Web site for investigation of skeletal biology. *L. Jia¹, M. Young², J. Powell³, J. Touchman⁴, G. Bouffard⁴, N.C. Ho¹, R. Hotchkiss⁵, P. Robey², C.A. Francomano¹.* 1) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Bone Resesarch Branch, NIDCR/NIH, Bethesda, MD; 3) CIT/NIH, Bethesda, MD; 4) NIH Intamural Sequencing Center, NIH, Bethesda, MD; 5) Hospital for Special Surgery, New York, NY.

The Skeletal Genome Anatomy Project (SGAP) is a multi-Institute project aimed at understanding the functional genome of bone and other skeletal tissues. SGAP is a resource under development through the collaboration of the Medical Genetics Branch, National Human Genome Research Institute (NHGRI), the Craniofacial and Skeletal Diseases Branch, National Institute for Dental and Craniofacial Research (NIDCR) and Bioinformactics and Molecular Analysis section of Center for Information and Technology (CIT) of National Institutes of Health, Hospital for Special Surgery (HSS) in New York City. It is intended to be a resource for scientists interested in both normal and abnormal skeletal growth and development. Toward that end a web site has been developed to provide information about skeletal-related cDNA libraries created and sequenced through SGAP and detail the methodologies utilized. The site is constructed to allow allow real-time data analysis of skeletal-related genes and ESTs, including assessment of expression patterns in skeletal tissues and other libraries in the NCBI databases. A Skeletal Gene Database is available at the Web site, listing genes and ESTs that play a role in skeletal development, their chromosomal location, cellular function, gene and transcript size and amino acid length of the protein product. Details are provided about skeletal-enhanced microarray resources, both filter and glass-based, developed through SGAP. Finally, the web site provides information about SGAP staff and collaborators, how to request reagents generated through SGAP, as well as links to other useful bioinformatics and genomics sites.

Cyberscreening for nuclear genes affecting mitochondrial gene expression. *D.P. Nierlich¹, S.A. Selahi¹, N. Hoang¹, D. Obukhov¹, Y. Bykhovskaya², T.W. O'Brien³, E.B. Mougey⁴, J.E. Sylvester⁴, H.-R. Graack⁵, B. Wittman-Liebold⁵, N. Fischel-Ghodsian².* 1) Microbiology & Molecular Genetics, UCLA, Los Angeles, CA; 2) Cedars-Sinai Medical Center and UCLA School of Medicine, Los Angeles, CA; 3) University of Florida, Gainesville, FL; 4) Nemours Children's Clinic, Jacksonville, FL; 5) Free University, & Max-Delbruck Center, Berlin, Germany.

Mitochondrial gene expression plays a clear role in a number of diseases as evidenced by mutations in the mt-DNA encoded components of mitochondrial protein synthesis, the mt-rRNAs and mt-tRNAs. Nonetheless, two features of these diseases are puzzling. Often they are confined to a single organ, and there is incomplete penetrance. To explain these features, it has been postulated that the mt RNAs interact with the nuclearly encoded components of mt-protein synthesis. The feature of tissue specificity has been postulated to be due to use of tissue-specific isozymes, perhaps generated by alternative splicing, and the penetrance may be due to allelic variation in these same genes (Mol Genet Metab 65:97, 1998). Thus to characterize the structural components of mitochondrial RNA processing and translation, the Mammalian Mitochondrial Ribosomal Consortium has been formed.

In this project we have searched human genomic- and cDNA-sequence libraries for homologs of experimentally established components of mitochondrial translation, primarily those of yeast. High scoring hits were then examined for a putative targeting sequence and for their phylogenetic relationships. Targeting was predicted using an algorithm that we developed based on the differential composition of the targeting sequences of mammalian mitochondrial-matrix proteins. Thus far, with this methodology, several previously unidentified human genes have been identified. In addition, comparisons to the TIGR THC database (www.TIGR.org) revealed a striking number of alternatively spliced human mitochondrial sequences.

This work was done as part of the Mammalian Mitochondrial Ribosomal Consortium and supported by NIH/NIDCD RO1DC04092.

A symbolic regression approach to mining gene expression patterns. *J.S. Parker, L.W. Hahn, J.H. Moore.* Program in Human Genetics, Vanderbilt University, Nashville, TN.

It is increasingly clear that our ability to simultaneously measure the expression levels of thousands of genes will revolutionize our understanding of the initiation, progression, and severity of human disease. However, progress in this area is dependent on the development and implementation of analytical methods that are able to identify gene expression patterns that are associated with a particular biological or clinical endpoint. We have developed an approach to identifying biologically or clinically relevant gene expression variables that combines the power of symbolic multiple regression (SMR) for pattern recognition and parallel genetic programming for machine learning.

SMR is a statistical and computational methodology for finding mathematical expressions, in symbolic form, that can take values of gene expression variables and produce biological or clinical endpoint predictions. SMR involves finding both the functional form and the numeric coefficients for a regression model. In contrast, traditional linear, quadratic, polynomial, and logistic multiple regression methods involve finding only numeric coefficients for a function that has been pre-specified. We have implemented parallel genetic programming for machine learning optimization of variable and SMR model selection.

Using simulated and real gene expression data, we have demonstrated that this approach can identify gene expression patterns that are associated with biological and clinical endpoints. Further, the SMR approach outperforms traditional methods such as cluster analysis and discriminant analysis when the gene expression patterns are complex. The SMR approach provides a powerful alternative to traditional statistical methods for identifying gene expression patterns.

Primary chromosomal rearrangements are frequently accompanied by extensive submicroscopic deletions in leukemias. *J.A. Squire, E. Kolomietz, J. Al-Magharbi, J. Karaskova, S. Minkin.* Dept Cellular & Molecular Biol, Ontario Cancer Inst, Toronto, ON., Canada.

During routine dual-color BCR/ABL fluorescence in situ hybridization (FISH) analysis of chronic myeloid leukemia (CML) cells we observed that a subset of samples exhibited an atypical hybridization pattern suggestive of deletion. A retrospective D-FISH study of 250 cases of CML patients in chronic and blast crises and 13 cases of Ph+ ALL patients indicated that 23 CML patient (9.2%) and 1 ALL patient (7.7%) exhibited a pattern consistent with deletion of the two regions: proximal to the rearranged ABL and distal to BCR gene on the 9q+ derivative chromosome. The deletions involve several hundred kilobases of DNA. This data are consistent with the simultaneous occurrence of deletions and structural rearrangements. The observation, that deletions are associated with both Ph-positive CML and ALL leukemias suggested that other leukemia-associated genomic rearrangements may also lead to the deletions. This notion was confirmed by the detection of the deletion of 3'region of CBFB gene in 20%(n=10) of the AML M4 patients with inv 16(p13q22). Clinical outcome data indicates that in both leukemias deletions correlates with poor prognosis. We postulate that such deletions can lead to loss of one or more tumor suppressor genes that map close to the breakpoints and that the associated haploinsufficiency may result in a modification of the disease phenotype in both leukemias. FISH analysis of the AML M3 group of patients (n=30) showed however that no deletions were associated with t(15;17) (q22;q21). Taken together, this observation suggests common underlying molecular mechanism that lead to the deletions associated with chromosomal rearrangements and Alu sequences that map close to the breakpoint regions may be implicated. We propose that Alu elements-mediated unequal homologous recombination may be responsible for the deletions associated with non-random chromosomal rearrangements. This hypothesis suggests that the mechanism of chromosomal rearrangements may be greatly influenced by local variation in sequences.

An automated system for sequence analysis, annotation, and comparison. *T.D. Taylor¹, H. Watanabe¹, K. Gun¹, J. Sun¹, Y. Sakaki^{1,2}.* 1) Human Genome Research Group, RIKEN Genomic Sciences Center, Sagamihara, Japan; 2) Human Genome Center, University of Tokyo, Japan.

Our center is sequencing regions of human chromosomes 11 and 18 (and most recently helped complete chromosome 21). We are also identifying new genes and functional units in the laboratory. While there are many individual tools for the analysis, annotation, management, and presentation of sequence data, there are only a few suites that combine a useful subset of these tools. Therefore, we are developing a portable system that includes the best features from these other packages as well as many new features, most notably the inclusion of cross-species data comparison.

Our system is built around a Perl/Tk interface, which can be used for conducting the analyses and visualizing the results. An XML database is used for data storage, allowing for subsequent statistical analyses and comparisons. Perl modules link everything together and perform some of the analyses, while externally written programs are tied in locally or over the WWW. Briefly, sequences are checked for errors and then piped through a user-specified set of programs. Result files are checked for validity and parsed. Duplicate or insignificant results are filtered out. The results are then be displayed graphically for evaluation. The user has the option to rerun some of the analyses using different parameters, run additional analyses, make annotations and export them for submission, etc. To gain maximum information from the data, multiple types of analyses can be run and compared. Some analyses (i.e., BLAST) can be set to automatically rerun after a specified time-period, and any new findings can be highlighted.

We are implementing a comprehensive system that is as customizable and extendable as possible, yet is still user-friendly and cross-platform compatible. The analysis of different genomes requires different subsets of analytical tools; our system allows for this flexibility and retains the same basic functionality for the user. Our system is also being designed to analyze sequences as automatically and quickly as possible.

Assessing reliability of gene clusters from gene expression data. *K. ZHANG, H. ZHAO.* Epidemiology & Public Health, Yale University School of Medicine, NEW HAVEN, CT.

The rapid development of microarray technologies has raised many challenging problems in experiment design and data analysis. Although many numerical algorithms have been successfully applied to analyze gene expression data, the effects of variations and uncertainties in measured gene expression levels across samples and experiments have been largely ignored in the literature. In this presentation, in the context of hierarchical clustering algorithms, we introduce a statistical resampling method to assess the reliability of gene clusters identified from any hierarchical clustering method. Using the clustering trees constructed from the resampled data, we can evaluate the confidence value for each branch in the observed clustering tree. A majority-rule consensus tree can be obtained showing clusters that only occur in a majority of the resampled trees. We illustrate our proposed methods with applications to two published data sets. Although the methods are discussed in the context of hierarchical clustering methods, they can be applied with other cluster-identification methods for gene expression data to assess the reliability of any gene cluster of interest.

Identification of biologically relevant genes in different tissue types measured by oligonucleotide microarrays and their use in sample classification. *D.J. Wilson, S. Peres-da-Silva, C.P. Alvares, D.T. Horne, T. Eskandari, A.J. Williams, L.M. Vogt, U. Scherf, J.G. Vockley.* Genomics R&D, Gene Logic Inc., Gaithersburg, MD.

As the complexity of gene expression information generated by microarray experiments increases, it becomes important to develop additional analysis methods that reliably determine biologically relevant genes. We have refined a strategy that systematically examines the gene expression patterns of different samples of an identical tissue type and identifies the profile of consistently expressed genes. These patterns of commonly expressed genes with their individual gene expression values were extracted from the raw expression data for each of three tissue sets. The gene expression pattern of nine myometrium samples was determined to be statistically similar to the pattern of an independent myometrium sample and different from the pattern of a colon or thymus sample. The same approach has been repeated for colon and thymus sample sets with similar results. As an application of these results, we predict that this method can be used to determine the tissue type identity of an unknown tissue.

High through-put annotation of the rough draft of the Human Genome. *N. Tsinormas*. DoubleTwist, Inc, Oakland, CA., USA.

The Computational Genomics Group, DoubleTwist Inc. The year 2000 will mark the completion of the first phase of the Human Genome Project. Using these data to further understand the genome creates several challenges. In this presentation, we will focus on DoubleTwists effort to provide a Web-based computational environment which greatly facilitates the interpretation of working draft human genome sequence data. The DoubleTwist site provides users with a Web-based system which allows easy and comprehensive analysis against all publicly-available human genome sequence data, whether finished or framework sequences. The data in this database have been pre-processed so as to mask sequences which are contaminated with foreign sequences from E.coli, yeast, vector, phage etc. Repetitive sequences are also masked . Using a sophisticated JAVA-based viewer, the user is able to visualize similarities to cDNA/ESTs, protein and protein domain signatures, as well as inferred gene structures, along with an alignment of the users query sequence against the BAC fragment. Users of DoubleTwist are provided with a mechanism that notifies them immediately via E-mail when a genomic sequence has been released which matches their interest profile, whether because it contains a gene of interest, or because it has recently been upgraded and newer versions of the sequence have been generated.

In silico approach for identification of SCA10 gene using eRED database from the whole genomic sequence data of the human chromosome 22. *H. Rhee*¹, *K. Lee*², *B. Oh*², *J. Lee*³. 1) Department of Molecular Medicine, College of Medicine, Yonsei University, Seoul, Korea; 2) Division of Genetic Diseases, Korea National Institute of Health, Seoul, Korea; 3) Department of Pediatrics, College of Medicine, Yonsei University, Seoul, Korea.

The spinocerebellar ataxia(SCA) result from unstable expansions of particular trinucleotide repeats. And especially, all the known genes associated with the SCA to date, i.e. SCA1, SCA2, SCA3, SCA6, SCA7, SCA8 and SCA12, result from the CAG/CTG repeat expansions. A new type of SCA, the SCA10, was recently mapped to the chromosome 22q by linkage analysis and the rather short triplet repeat expansions have been postulated to be involved in the pathogenesis. Since the whole genomic sequence data of the human chromosome 22 is now available, it might be able to hunt for the SCA10 gene by searching the triplet repeat region from the whole sequence data of the human chromosome 22. From this hypothesis an eRED (electronic repeat expansion detection) database was constructed containing all genomic region in which the copy numbers of the CAG/CTG repeats are greater than 3. We searched the whole genomic sequence data of the human chromosome 22 for the CAG/CTG string with the locally developed program eRED-KMP which utilizes the Knuth-Morris-Pratt algorithm for the fast detection of the repetitive string. Then all the regions which contain the CAG/CTG sequences was compared with the Sanger Center's annotation for the putative genes on the chromosome 22. And the BLAST search between the annotated mRNA sequences and the unique sequences library from the human UniGene (Build 108) dataset to find the putative expression spectrum of the identified CAG/CTG repeats. The (CAG)₁₃ found at the base position 4,497,629 and (CTG)₁₃ at 7,608,654 were the longest repeats and there were also 7 (CAG)_n and 5 (CTG)_n regions that contain more than 7 repeats. Since the locus for SCA10 gene has been mapped between 25Mb-30Mb regions of the human chromosome 22q, 6 sequence tags containing variable numbers of CAG/CTG repeats identified within this region can be tested as strong candidates for SCA10.

Identification of candidate genes that control the peak bone density by combination of cDNA microarray analysis and physical mapping. *W.K. Gu¹, X.M. Li¹, S. Mohan¹, K-H.W. Lau¹, B. Edderkaoui¹, L.R. Donahue², C. Rosen³, W.G. Beamer², D.J. Baylink¹.* 1) Musculoskeletal Disease Ctr, Loma Linda Univ & JL Pettis VAMC, Loma Linda, CA; 2) The Jackson Lab., Bar Harbor, ME; 3) St. Joseph Hosp., Banger, ME.

Osteoporosis is a debilitating bone disease that afflicts 30 millions people in the U.S. Peak bone density is an important determining factor of future osteoporosis risks. Genetic factors determine up to 80% of variation in peak bone density. Our previous study has identified a QTL locus on mouse chromosome 1 that contributes to 40% of the total peak bone density difference between C57BL/6J (B6) and CAST/EiJ (CAST) mouse strains. We have produced congenic mice in which the chromosome 1 fragment containing this QTL locus has been transferred from CAST to the congenic mouse strain of B6 background. To identify candidate genes within this QTL locus, we have developed an approach in which we combined physical mapping and cDNA microarray analysis to select candidate gene(s) based on their relative expression level and chromosomal locations. In this study, we constructed a BAC contig that contains 40 BAC clones and covers the peak region of this QTL locus (92 to 94 cM) on mouse chromosome 1. cDNA microarray analysis was conducted by Incyte Genome Systems. The isolated mRNA from femur of both the congenic and B6 strains were labeled with different dyes and hybridized onto the same microarray chip containing 8,739 genes and ESTs. This analysis revealed that 104 genes and ESTs showed at least 2-fold difference between the two strains. Among these candidate genes, 19 are known genes and 85 are ESTs. Among the known genes, 10 have functions relevant to bone. Only one (petaxin-related C-reactive protein) is located within the chromosome 1 QTL region. Among the ESTs, three are matched to the QTL locus of chromosome 1 and two are located within our BAC contig. In summary, we have identified 3 peak bone density candidate genes (C-reactive protein and 2 ESTs) in mice by our combination approach of physical mapping and cDNA microarray analysis. [This work was supported by USAMRAA through Assistance Award No. DAMD17-99-1-9571].

Serial analysis of gene expression of the human sciatic nerve. *R.R. de Jonge, J.P. Vreijling, I.N. van Schaik, F. Baas.* Neurozintuigen Laboratory, Academic Medical Center, Amsterdam, The Netherlands.

The analysis of genes involved in synthesis and maintenance of peripheral nerve myelin has, thus far, mainly been focused on pathological. To get a better understanding of the physiological process of myelination and the molecular pathways involved, we performed serial analysis of gene expression (SAGE) of the human sciatic nerve. This technique allows rapid and detailed characterization of gene expression patterns. Human sciatic nerve was obtained post-mortem and SAGE was performed using the standard protocol by Velculescu. The sequencing of 1650 clones yielded a library containing 20287 tags, representing 9422 different genes. Northern blot analysis performed for a number of genes confirmed the expression levels found by SAGE. Our results show high expression of peripheral nerve-specific genes, such as myelin basic protein, apolipoprotein D, protein zero, myelin-associated glycoprotein, glutathione peroxidases, zinc-finger proteins and S100 calcium-binding proteins. Moreover, high expression of factors of the complement system were found, as well as insulin-like growth factor binding proteins, matrix metalloproteinase and proteo-glycans. Functional studies have to be performed to elucidate the role of these genes in the peripheral nervous system, and their possible role in myelinating disorders.

YAC/BAC contig and STS mapping of the human GABA_A receptor subunit gene cluster on chromosome 4. *J. Du, S.A. Karim, K.J. Johnson, M.E.S. Bailey.* Division of Molecular Genetics, I.B.L.S., University of Glasgow, Glasgow, U.K.

The GABA (g-aminobutyric acid) type A receptor is a ligand-gated ion channel complex that is important in inhibitory neurotransmission in the vertebrate brain. Each of the 19 distinct related subunits known in mammals, which are classified into seven subclasses (a, b, g, d, e, q, p and r), is encoded by a separate GABR gene. A cluster of genes, *GABRA2*, *GABRA4*, *GABRB1* and *GABRG1*, encoding 4 subunits (the $\alpha 2$, $\alpha 4$, $\beta 1$, and $\gamma 1$ subunits, respectively) is located on human chromosome 4p12-p13. We are interested in understanding the influence of regulatory sequences within and between the genes on control of gene expression in the cluster. We have characterised a large insert clone contig from which to identify these regulatory regions.

From published studies and our own library screening, we have identified an incomplete YAC clone contig that spans the chromosome 4 GABR gene cluster and several BAC clones containing the 5'- and 3'-ends of the four genes. We have used known STS markers, novel clone-end STS markers generated by sequencing and by vectorette PCR, and inter-Alu PCR to construct a detailed STS map of the region containing the entire gene cluster. At this stage of the human genome sequencing project, database searching has revealed an additional 6 BAC clones at the rough draft sequence stage that contain parts of the four genes as well as several of the STSs and we have been able both to incorporate these into the contig and to use them to refine the marker order.

The STS map establishes that the order of genes in the GABR gene cluster is 4pter-*GABRG1*-*GABRA2*-*GABRA4*-*GABRB1*-4cen. The first three of these are arranged in head to tail fashion with their 5'-ends closest to the centromere, while *GABRA4* and *GABRB1* are arranged head-to-head. A gap in the contig occurs immediately telomeric to *GABRA4*. Our results are consistent with previous studies and suggest that the structure of the cluster is conserved with that of the other human GABR gene clusters. The contig provides the basis for an investigation of the regulation of genes in the chr.4 cluster.

Folate metabolism and meiotic recombination: interlocking pieces of the nondisjunction puzzle. *N.E. Lamb¹, P. Yi², R. Rozen³, C. Hobbs⁴, S.J. James⁴*. 1) Genetics, Emory Univ, Atlanta, GA; 2) FDA-National Center for Toxicological Research, Jefferson, AR; 3) McGill Univ., Montreal Children's Hospital Research Institute Montreal, Quebec, CA; 4) Arkansas Center for Birth Defects Research, Little Rock, AR.

In oocytes, altered chiasmata placement has been identified as a risk factor for chromosome 21 nondisjunction (NDJ). This risk creates a susceptible oocyte that, in the presence of additional factors that disrupt meiosis, can undergo NDJ. Recently, polymorphisms in two folate metabolism genes, methylenetetrahydrofolate reductase (MTHFR:C677T) and methionine synthase reductase (MTRR:A66G), have been implicated to increase the risk of NDJ in mothers (e.g., OR of mothers with CT/TT and GG compared to CC and AA/AG=4.08; 95%CI=1.94-8.56). These high risk genotypes may result in chromosome hypomethylation, known to perturb mitotic processes. A similar effect may occur in meiosis. Coupled with susceptible chiasmate configurations, altered folate metabolism may significantly increase NDJ risk. To test this idea, we compared frequencies of MTHFR and MTRR high risk genotypes among mothers with a maternal meiosis I NDJ (MI) error grouped on the basis of recombination along the nondisjoined chromosomes 21. We found a significantly increased frequency of high risk genotypes among mothers with recombination compared to those with none ($p<.05$). To identify any increased risk based on both recombination and altered folate metabolism, we then compared these MI mothers to a set of control mothers without NDJ. When compared to controls, mothers with no recombination did not have an increased frequency of susceptible folate genotypes. In contrast, we found a significantly increased OR for those with recombination (OR 10.19; 95%CI=2.51-41.41). Such data are compatible with a multi-step model for nondisjunction. Achiasmate oocytes, already at great risk for NDJ, need no other risk factors. Oocytes with susceptible chiasmata placement however, require additional risk factors as a second step. Abnormal folate metabolism may serve as such a factor. Currently, we are analyzing 150 additional trisomy 21 cases to further refine our observations.

Developmental Genome Anatomy Project: Two Breakpoints Localized in BAC clones. *E. Lemyre*^{1, 5}, *G.A.P. Bruns*^{3, 5}, *J.F. Gusella*^{2, 5}, *B.R. Korf*^{1, 2, 5}, *S.R. Herrick*¹, *A.H. Ligon*^{1, 5}, *J. Lewis*^{2, 5}, *R.L. Maas*^{1, 5}, *M.E. MacDonald*^{2, 5}, *A.M. Michelson*^{1, 4, 5}, *B.J. Quade*^{1, 5}, *C.C. Morton*^{1, 5}. 1) Brigham & Women's Hospital, Boston, MA; 2) Massachusetts General Hospital, Charlestown, MA; 3) Children's Hospital, Boston, MA; 4) Howard Hughes Medical Institute; 5) Harvard Medical School, Boston, MA.

The goal of the Developmental Genome Anatomy Project (DGAP) is to identify and study genes critical in human development by analyzing samples from patients with balanced chromosomal rearrangements and at least one congenital anomaly. We hypothesize that in a substantial proportion of these patients a developmentally important gene will be disrupted or dysregulated by the breakpoint and be etiologic in the abnormal phenotype. The incidence of apparently balanced chromosomal rearrangements is estimated to be 1/2000 newborns. Six-9% of these, at least twice the rate of the general population, will carry an abnormal phenotype. DGAP involves three steps: FISH mapping of the breakpoints using BACs at ~1 Mb intervals; sequence analysis and candidate gene identification in the region of the breakpoints; and functional analysis of candidate genes in model organisms (mouse and *Drosophila*). During the last year we have established a growing network of collaborators among clinical cytogeneticists, clinical geneticists and genetic counselors around the United States and abroad. To date, we have collected 79 samples from patients with a variety of developmental defects and apparently balanced rearrangements. According to a prioritization scheme, we FISH-mapped eight breakpoints at the highest resolution possible using a genomic BAC map. Two of these eight breakpoints have been localized to single BACs. Molecular analyses of these cases are underway. To date, no micro-rearrangements at the site of individual breakpoints have been found. The DGAP website (<http://dgap.harvard.edu>) posts clinical and cytogenetic data and tracks the progress for cases in our database. We believe this will be a powerful resource for scientists in the genetics and developmental biology communities.

Additional complexity on the long arm of human chromosome 15: a 2 Mb bacterial clone-based contig from 15q25.3 to 15q26.1. *M.A. Pujana, M. Nadal, M. Gratacos, B. Peral, L. Sumoy, X. Estivill.* Medical and Molecular Genetics Center, Cancer Research Institute, Barcelona, Spain.

Several cytogenetic alterations affect the distal part of the long arm of human chromosome 15, including recurrent rearrangements between 12p13 and 15q25, which cause congenital fibrosarcoma (CFS). We present here the construction of a BAC/PAC contig map that spans 2 Mb from the neurotrophin-3 receptor (NTRK3) gene region on 15q25.3 to the centromeric end of the Bloom's disease region on 15q26.1. The contig reveals the existence of several regions of similarity with other chromosomes (6q, 7p and 12p) and with other 15q cytogenetic bands (15q11-q13 and 15q24). The relative distances between the respective regions of similarity and ets variant 6 (ETV6) gene on 12p and NTRK3 gene on 15q26.1 are equivalent, suggesting that misalignments between these two chromosomes could facilitate recombination events, leading to CFS. The 15q11-q13 region of similarity is located close to the Prader-Willi/Angelman syndromes imprinting center. The 15q24 similarity is due to the presence of low copy repeat sequences that span about 26 kb on 15q24 and about 22 kb on 15q26.1. Different copy numbers of these low copy repeat sequences are present along 15q: 15q24 and 15q26.1. As for several human genomic disorder mutations, low copy repeats are the flanking elements present in chromosome regions prone to reorganizations, giving rise to deletions and reciprocal duplications through non-homologous recombination events. The 15q repeated regions described here could explain some reported chromosomal rearrangements involving the distal portion of this chromosome arm, or they could even justify the identification of new genomic mutations associated to these regions.

Multicolor spectral analysis of the rat karyotype using the homologous murine SKY™ chromosomal probe. J.

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Recently rodent models of human oncogenesis have provided valuable information on the early genetic changes associated with neoplasia. The murine gene map is well established and various multicolor painting techniques such as Spectral Karyotyping (SKY™) have helped with the analysis of tumors arising in the mouse. The most widely studied mammalian model of cancer is the rat and therefore cytogenetic analysis of this karyotype is frequently required. The absence of a rat chromosome-specific paint set limits our ability to perform detailed rat molecular cytogenetics. Conventional G-banding analysis of rat metaphases is time consuming and there is no experience base for detecting small aberrations with a high degree of certainty. To address this deficiency and to develop SKY analysis in the rat we applied the commercial mouse SKY™ probe kit to rat metaphase chromosomes on the basis that there would be sufficient phylogenetic conservation between these closely related rodents for reproducible multicolor analysis. The murine SKY probe hybridized to rat chromosomes creating specific color-banding patterns on each rat chromosome. Each murine chromosome was assigned one specific color thus it was possible to compare each mouse chromosomal origin for the majority of the rat karyotype. The derived cytogenetic data were in good agreement with comparative gene mapping data and reciprocal FISH chromosome painting between mouse and rat published previously (Stanyon et al., 1999). In conclusion "rat SKY" allows for the simultaneous identification all rat chromosomes and can be readily evaluated using the visible RGB segmented color-display banding pattern. The computer-classified colors assignments based on mouse chromosomal origin together with the inverted DAPI pattern banding provide a high degree of certainty of subchromosomal regional identity. We propose that using rat SKY both the numerical and structural chromosomal changes in rat can be readily detected and their precise origins can be determined.

Identification of sequences at an evolutionary breakpoint and an inversion through comparative mapping of human 19p13.3 and mouse Chromosome 10. *M. Burmeister¹, R. Puttagunta¹, L.A. Gordon², G.E. Meyer¹, D. Kapfhamer¹, J.E. Lamerdin², P. Kantheti¹, K.M. Portman¹, W.K. Chung³, D.E. Jenne⁴, A.S. Olsen².* 1) Mental Health Research Inst., Depts. of Psychiatry and Human Genetics, Univ. of Michigan, Ann Arbor, MI; 2) Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA; 3) Columbia Univ., New York; 4) Max Planck Inst. of Neurobiology, Martinsried, Germany.

A cosmid/BAC contig map of HSA 19p13.3 has been constructed, and over 50 genes have been localized to the contig. Genes and anonymous ESTs from 4000 kb of human 19p13.3 were placed on the central mouse Chromosome 10 map by genetic mapping and PFGE analysis. A region of about 2500 kb of HSA 19p13.3 is collinear to MMU 10. In contrast, the adjacent ~1200 kb are inverted. Two genes are located in a 50 kb region after the inversion on MMU 10, followed by a region of homology to mouse Chromosome 17. The synteny break point and one of the inversion break points have been localized to sequenced regions in human < 5 kb in size. Both breakpoints are rich in simple tandem repeats, including (TCTG)_n, (CT)_n and (GTCTCT)_n, suggesting that simple repeat sequences may be involved chromosome breaks during evolution. These sequences will be compared to the emerging sequences of the human genome near other synteny break points.

The overall size of the region in mouse is smaller although no specific genes or regions are missing. Comparing the physical maps to the genetic maps showed that in contrast to the higher than average rate of genetic recombination in gene-rich telomeric region on HSA 19p13.3, the average rate of recombination is lower than expected in the homologous mouse region. This might indicate that a hot spot of recombination may have been lost in mouse or gained in human during evolution, or that the position of sequences along the chromosome (telomeric compared to the middle of a chromosome) is important for recombination rates.

Comparative mapping of human chromosome 14q11.2-q13 genes with mouse homologous gene regions. D.

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An examination of the synteny blocks of homologous genes between mouse and human chromosomes aids in understanding the evolution of chromosome divergence between these two species. Based on current information of physically mapped genes on human chromosome 14, the gene content and order demonstrate conservation of synteny with genes mapping to mouse chromosomes 12 and 14. In addition, more than two thirds of human chromosome 14, that is, from 14q12-qtel, exhibits almost continuous synteny with mouse chromosome 12, with the exception of *BMP4*, *GCH*, *KTN1* and *OTX2*, at 14q21-q23, which have orthologues that map to mouse chromosome 14. We comparatively mapped the human chromosome 14q11.2-q13 cytogenetic region with the intervals of orthologous genes on mouse chromosomes. A lack of conserved gene order was identified between the human cytogenetic region and the interval of orthologues on mouse chromosome 12. The evolutionary breakpoint junction was defined within 2.5 Mb, where the conserved synteny of genes on human chromosome 14 changes from mouse chromosome 12 to mouse chromosome 14. At the evolutionary breakpoint junction, a human EST (GI:1114654) with identity to the human and mouse BCL2 interacting gene, *BNIP3*, was mapped to mouse chromosome 3. New gene homologues were identified on the proximal cytogenetic region of human chromosome 14 and human chromosome 7, by mapping mouse genes recently reported to be genetically linked within the relevant mouse chromosome interval. This study contributes to the identification of homology relationships between the genes of human chromosome 14q11.2-q13 and mouse chromosomes 3,12 and 14. In addition, the newly mapped genes on the proximal region of human chromosome 14 serve as candidates for disorders mapping to this region.

Initiation of a chimpanzee genome project utilizing a 10kb total genomic library and the human genome draft sequence. *E. Nickerson, R.A. Gibbs.* HGSC, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Chimpanzees represent the closest extant species to *Homo sapiens*. The genomes of humans and chimpanzees have been shown to be >98% identical by DNA sequence and DNA-DNA hybridization analyses. The two primates are, however, undeniably distinct at the phenotypic level. Comparative sequence analysis to date, predominantly in coding regions, has primarily served to emphasize the identity between the genomes. Thorough characterization of the <2% difference between the human and chimpanzee genomes will ultimately lead to the identification of genetic changes that were integral to the divergence of the species. Much can be learned about trends in total genomic dynamics and the consequences of genetic change to speciation if the human and chimpanzee genomes are compared in their entirety.

We have initiated an effort to sequence the chimpanzee genome. A chimpanzee total genomic library with 10kb inserts of *Pan troglodytes* DNA is under construction. Double stranded sequencing reads will be performed to the resolution of 1-2X coverage of the entire genome. The human genome draft sequence will be used as a scaffold for sequence assembly. By this approach, significantly divergent regions of the genome will be easily identifiable. Regions showing increased nucleotide divergence (>2%) and species-specific differences in the copy number of low-copy repeats and coding sequences will be identified, further sequenced at higher resolution and evaluated as potential contributors to speciation. Breakpoints of gross chromosomal rearrangements known to distinguish the species karyotypes as well as rearrangements beyond the resolution of cytogenetics, such as microdeletions and small paracentric inversions, will also be appraised.

The draft sequences of the human and chimpanzee genomes will be valuable reagents for the identification of genomic changes that may have been critical to the evolution of the species. Furthermore, the chimpanzee genome project will provide a valuable tool to expedite higher primate comparative analysis for individual researchers with specific regions of interest.

A 700 kb contig of sequence-ready bacterial clones from mouse chromosome 1H spanning the syntenic region for the Van der Woude syndrome locus at human chromosome 1q32-q41. *B.C. Schutte¹, M.I. Malik¹, S. Gregory², M. Fang¹, J.C. Murray¹.* 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Sanger Center, Hinxton, UK.

Van der Woude syndrome (VWS) is an autosomal dominant orofacial clefting disorder (OMIM #119300). The VWS locus maps to a 1.6-cM region between markers D1S491 and D1S205 on chromosome 1q32-q41. Based on analysis of 1 Mb of genomic sequence, the VWS critical region is 350 kb in length and contains at least 13 genes. We initiated a comparative genomic approach to identify additional genes in this region. As a first step, a contig of sequence-ready bacterial clones was constructed across the syntenic region of mouse chromosome 1H. Gene-specific STSs were generated from the partial sequence of 11 mouse genes whose human ortholog maps near the VWS locus. These STSs were used to identify 17 bacterial clones from the CT7 BAC library. An additional 11 STSs were generated from the end sequence of these clones. STS content analysis indicated that these clones formed a partial contig across the region with a single gap. To complete the contig, the RP23 BAC library was screened using all 28 STSs. A total of 48 clones were identified. Hybridization, fingerprint and STS content analyses demonstrated that these clones formed a complete contig. The order of the genes in the mouse contig (TEL-Vws1-Lamb3-G0s2-Hsd11-Vws17-Vws8-Vws2-Vws18-Vws30-Vws35-Vws31-CEN) is identical to the order on human chromosome 1, except the centromere-telomere orientation is reversed. This approximately 700 kb contig of sequence-ready clones provides the resources to identify new genes at the VWS locus using comparative genomic analysis and significantly expands our understanding of the syntenic relationship between human 1q32-q41 and mouse 1H.

An automated method for inferring DNA sequence variation from high density oligonucleotide arrays. *D. Cutler¹, M. Zwick¹, C. Kashuk¹, D. Mathews¹, D. Nelson³, R. Gibbs³, N. Shah², J. Warrington², E. Eichler¹, A. Chakravarti¹.* 1) Department of Genetics, Case Western Reserve Univ., Cleveland, OH; 2) Affymetrix Inc. San Jose, CA; 3) Baylor College of Medicine, Houston, TX.

High density oligonucleotide arrays, produced by a variety of technologies, offer the promise of providing DNA variation data from multiple individuals in an efficient and rapid manner. The array data assayed as a fluorescence signal at each possible base, pose a unique analysis challenge: can millions of DNA basepairs be inferred in a reliable, objective and repeatable manner without human intervention? One method to make these inferences is to statistically distinguish bases for which sufficient fluorescence information is present to make reliable, repeatable base calls, from those sites with insufficient data. To do so, a set of probability models is developed. For haploid data four models are considered, one for each of the possible nucleotides {A,C,G,T}. For diploid data, these same four models are examined, as are six additional models, one for each of the possible heterozygotes {(A,C),(A,G),(A,T),(C,G),(C,T),(G,T)}. Observed fluorescences are assumed to be normally distributed, but other distributions can be utilized. Model parameters are estimated from the data using maximum likelihood, and if one model fits the data significantly better than all alternative models, those bases are called; otherwise, no inference is made. Two types of experiments were conducted to validate this approach on custom high density arrays manufactured by Affymetrix Inc. The first, a replication experiment, consisted of independent long-PCR amplification of genomic DNA, independent manufacture, and hybridization of amplified DNA to GeneChip microarray. This experiment was carried out on the X-linked FMR1 locus from 32 males. In total, 829,610 bases were called identically. The replicate experiment was also performed on autosomal data. 799,727 genotypes (including 127 heterozygotes) were called identically, but six genotypes were called differently. In a second experiment to verify accuracy, a single (FMR1) individual was shotgun sequenced to 6X coverage. All 17,876 bases identified were identical to array calls.

Beyond BLAST: Paleogenomics Tools to Infer Function to Genetic Sequences. *S. Benner, S. Chamberlin.* EraGen Biosciences, Inc, Alachua, FL.

While genomic sequence data are widely believed to hold the key to a revolution in biology, much of the revolution has not yet materialized. A central obstacle are difficulties converting DNA sequences (which are no more than chemical structures for organic molecules) into information that can be used by a biologist. This process is known as "annotation". Presently available annotation tools are well known not to provide their user with the information that they need to formulate good hypotheses regarding the function of a gene identified as an open reading frame. Many of the "evolution-based" strategies are now known to generate incorrect inferences. This talk will outline a suite of tools, including the EraGen Master Catalog, that organize and analyze genomic sequence databases using rigorous and sophisticated evolutionary models. These tools offer powerful approaches to some of the most difficult tasks in genomics, including the organization and retrieval of sequence data, updating of massive genomic databases, detection of database error, prediction of protein fold from protein sequences, detection of distant homologs, correction of annotation errors, assignment of function to open reading frames, identification of biochemical pathways from genomic data, and identification and validation of pharmaceutical targets. The talk will provide examples from mammalian biology and human therapeutics where bioinformatics generates hypotheses about physiology and function that are then tested by experiment. These include leptins (obesity gene proteins), aromatases (which make estrogen), phospholipases (involved in second message generation) and protein tyrosine kinases (involved in signal transduction). These show how a "natural database organization" is pivotal to realizing the value offered to biology by genomics.

Evaluation of an analysis tool for comparative DNA sequencing analysis and mutation detection. *L. Johnston-Dow¹, G. Chappell¹, H. Breu¹, S. Kumar¹, P. Suri¹, L. Brown¹, G. Mason¹, B. Iasnopolski¹, B. Kshirsargar¹, R. Paul¹, D. Siu¹, P. Honebein¹, E. Sword², M. Schoppe², A. Bone², J. Nimmer², V. Bawge³.* 1) Informatics, Genetic Analysis, Applied Biosystems, Foster City, CA; 2) Group Logic Arlington, VA; 3) Systems Integration Solutions San Francisco, Calif.

Various software tools are available for the analysis and interpretation of de-novo DNA sequencing data. While such applications work well for sequencing for discovery many of the underlying assumptions in these tools are not valid when applied to comparative sequencing data. We have developed a new application for comparative DNA sequencing analysis. This tool uses procedures and algorithms better suited to the unique nature of comparative sequencing data. Specifically, the prior knowledge of a reference sequence available in many comparative sequencing projects is used to streamline and automate portions of the analysis. In addition, new algorithms were developed that leverage the high degree of sequence similarity found in comparative sequencing applications to aid in the analysis. Here, we present the results from the evaluation of this new software tool. Two datasets were used to assess the performance and quality of the results. Data from the sequencing analysis of the HIV Protease and the Reverse Transcriptase regions of 100 clinical samples was used. Analysis included basecalling and assembly of data from 7 sequencing primers into a consensus for each sample. Each 1250 bp consensus was compared to an HIV standard strain (HXB2) and to a panel of known HIV mutations and polymorphisms. Data from the sequencing analysis of the HLA-DRB region from 50 blood samples also was evaluated. This analysis requires the pairing of forward and reverse sequence reads from each specimen, derivation of the 270bp DRB1 exon 2 consensus sequence followed by the comparison of that consensus to a panel of allele specific nucleotide positions. For these analyses, this new application produced results concordant with those previously obtained using standard DNA analysis tools. In both cases the analyses were completed in significantly less time and required less manual manipulation of the data.

Some Observations on Identifying Disease-Related Genes Using Expression Data. *A.D. Smith, J.M. Satagopan, M. Gönen.* Epidemiology and Biostatistics, Mem Sloan Kettering Cancer Ctr, New York, NY.

We analyze the leukemia data (Golub et al., 1999) to identify a subset of genes that are best related to a binary disease endpoint AML vs. ALL. The data consist of expression levels from 7129 genes on each of 38 leukemia patients (27 ALL and 11 AML). The performances of the two-sample t-statistic and Golub et al's related G-statistic (group mean difference / sum of standard deviations) are compared using 3 different methods of optimal gene subset selection. The first method involves significant gene subset selection after adjusting for multiple comparisons (resampling-based for t or step-down Bonferroni for G). A total of 40 genes from the t-statistic and 59 from the G-statistic are significant at the 5% level. These two subsets share 39 genes. The second method compares the classification rates of the best genes identified by either metric using a Fishers discriminant function. The top 50 genes are separately found by ranking the magnitude of the t or G-statistic. We then examine the ability of several subsets of these 50 genes to correctly classify a validation sample of 34 patients (20 ALL, 14 AML). The top 2 and 7 genes from t and G-statistics respectively give maximum classification rates of 88% and 91%. The third method employs a Gap statistic (Hastie et al, 2000). For each subset size, this statistic quantifies the difference between the observed variation explained and that under the null hypothesis of no gene/disease association. The subset with the largest Gap statistic is declared optimal. Subsets of 1 through 50 top genes identified by each of t and G-statistics are evaluated by this method. The maximum Gap is observed for the top 4 genes identified by the t-statistic and the top 3 identified by the G-statistic.

The gene subset selected by the t-statistic as having the maximum classification rate is among those with near-optimal Gap estimates, while that selected by the G-statistic has a markedly suboptimal Gap estimate. Further, regardless of the choice of t or G-statistic, a multiple comparison correction results in a larger subset of genes than is necessary to obtain (a) the best classification rate, or (b) the maximum observed Gap.

Identification of the authentic full length amino acid sequence of Krit1 (CCM1) utilizing a combination of computational gene-prediction tools and RT-PCR. *T. Sahoo*¹, *I. Serebriiskii*², *E. Kotova*², *J. Peloquin*³, *E. Golemis*², *E.W. Johnson*³, *D.A. Marchuk*¹. 1) Dept Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Fox Chase Cancer Center, Philadelphia, PA; 3) Barrow Neurological Institute, Phoenix, AZ.

The Cerebral Cavernous Malformation type 1 (CCM1) gene product, Krit1, was originally identified in a yeast two-hybrid screen as a specific interacting partner for Rap1A protein, a member of the RAS family of small GTPases. Since then it has been shown that truncating mutations in the Krit1 gene are found in the majority of CCM1 families. However, difficulties with recombinant Krit1 protein expression and other incongruities suggested that the published amino acid sequence might be incomplete. Computational analysis of genomic sequence encompassing the region containing Krit1 using gene-prediction programs identified a number of putative exons, with homology to overlapping ESTs, upstream of the 5' end of the published Krit1 cDNA sequence. RT-PCR and 5' RACE experiments confirm that there are at least 4 additional exons encoding Krit1, resulting in more than 200 additional amino acids upstream of the originally described ATG translational start site. In addition, we have identified a novel frame-shift mutation in one of the newly identified exons of Krit1 in a CCM1 family. These data extend and establish the authentic Krit1 amino acid sequence, and suggest that the additional Krit1 exons may harbor mutations in other CCM1 families that have yet to show a Krit1 mutation. More significantly, these results substantiate the high degree of reliability of computational gene identification tools, and their value in identifying novel genes within raw, uncharacterized human genomic sequence.

Construction of a Human Chromosome 1 Physical and Transcription Map. *S.G Gregory.* The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, England.

The Sanger Centre has been funded to generate the complete map and sequence of human chromosome 1. The strategy of generating a chromosome specific sequence map provides a means whereby the international collaboration to determine the sequence of the whole genome can be coordinated, along with the development of a physical and transcription map in association with the chromosome 1 community.

The physical map is being built using landmark based mapping and restriction digest fingerprinting. 5486 sequence tagged sites (STSs), 88% RH mapped, have been used to identify 30700 PAC or BAC clones. Fluorescent restriction digest fingerprinting of 23000 clones and incorporation of 14000 clones from the GSC, St Louis, has produced an estimated 95% (227Mb) sequence contig coverage of the heterochromatic region of chromosome 1. Gaps between contigs are sized using fibre FISH and closed by (i) the generation of *de novo* STSs from clones at the ends of contigs or (ii) using end sequences to probe large insert clone libraries.

A 'working draft' of chromosome 1 is being produced as part of the Human Genome Project. The working draft comprises genomic sequence of each bacterial clone in the physical map, determined at an average 3X depth of coverage. The draft sequence is an intermediate step prior to the 'finishing'. Finishing involves the generation of additional shotgun sequence, when necessary, and directed additional sequencing and checking to close all gaps and resolve ambiguities. Sequence produced from a finished clone provides sequence accuracy of >99.99%. As of 5th of June, the Sanger Centre has generated 131Mb of draft sequence and 29Mb of finished sequence.

The Sanger Centre chromosome 1 project is providing a detailed manual annotation and experimental analysis on 'finished' sequence clones. Genomic sequence analysis incorporates both *in silico* gene prediction and experimental homology screening. To date, analysis of finished chromosome 1 genomic sequence has identified 433 genes from 256 finished PAC/BAC sequences. Sanger Centre chromosome 1 mapping, sequence and analysis data is released freely in the public domain via, <http://www.sanger.ac.uk/HGP/Chr1/>.

Functional mapping of mouse chromosome 11 (33-80 cM). *M.A. Pershouse, W.M Di, E.K. Brundage, A.C. Chinault.*
Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Even with the completion of a draft version of the human genome sequence only a fraction of the genes identified from this sequence have known functions. Genetically engineered knockout mice, in concert with gene replacement assays to prove the functional significance of a given genomic region or gene, represent a rapid and productive means for understanding the role of a given set of genes. Both techniques rely heavily on detailed maps of chromosomal regions, initially to understand the scope of the regions being deleted, and finally to provide the cloned resources necessary to allow both finished sequencing and large insert complementation. This report describes the creation of a BAC clone contig on mouse chromosome 11. The region described is part of a large effort to create a series of knockouts encompassing the region of mouse chromosome 11 conserved by synteny with human chromosome 17 (33-80 cM). We have created a detailed, but as yet incomplete, map of this region through the use multiplex oligonucleotide hybridization and gap filling. Up-to-date maps of the 33-80 cM region will be presented.

A chromosome 18-specific gene array based on trapped exons: implications for bipolar disorder. *H. Chen, C.A. Ross, J.R. DePaulo, M.G. McInnis.* Johns Hopkins University, Baltimore, MD.

Human chromosome 18 (HC18) has been demonstrated to contain susceptibility genes for bipolar disorder. To identify the genes with potentially altered expression patterns in bipolar disorder, we created a gene array using a set of 1200 unique trapped exons. These exons were isolated from HC18-specific cosmids with exon trapping. There were 122 exon from 41 of the 80 known genes of HC18, 161 identical to ESTs, 49 with significant homology to genes from human and other species; the remaining 868 exons are novel. Search of the GenBank htgs-database indicated that 70% of the 1200 exons were identical to regions of HC18-genomic sequences available (6/12/00). Allowing for an average of 3 exons for one known gene (122/41), these exons may represent 400 genes. To further study the gene content of HC18, computer algorithms were used to predict genes within finished and working draft sequences. MZEF was used to predict internal exons and experienced an output of 25-50 "exons" per 200 kb; while GenScan was used to predict ORF's. We have analyzed the 23 finished sequence contigs on HC18 (6/12/00) and predicted over 650 exons by MZEF, and 100 ORF's in the 23 sequences. The BLAST programs are being used to compare sequences between trapped exons and predicted "genes" to produce a set of predicted "genes" unique to the trapped exons. RT-PCR analysis is being used to amplify the predicted "genes" from several tissues include brain, heart, kidney, liver. The set of predicted "genes" amplified with RT-PCR forms an additional portion of the HC18-gene array.

Identification of Potential Drug Targets using Gene Expression Data: A Statistician's Perspective. *F. Gheyas.*
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Pharmaceutical industries are focusing tremendous amount of research efforts and resources to discover drug targets using genomics. One of the popular approaches is to profile RNA expression levels of genes in cell lines and tissues from diseased versus healthy individuals. Genes that tend to show differential expression in diseased tissues may become potential drug targets. Furthermore, expression profiling may be used in proper classification of diseases. Recent progress in human genome sequencing and development of oligonucleotide arrays and cDNA microarrays has made it possible for scientists to simultaneously study the expression levels of thousands of genes. This has led to the creation of huge databases. These databases need to be analyzed using proper statistical methods. For the past few years, statisticians have been trying to develop new techniques and modify existing techniques to address issues specific to gene expression databases. These issues include experimental designs, normalization of data from different microarrays/chips, separation of signal from noise, and appropriate methods to group genes and tissue samples. Application of some of these statistical methods will be demonstrated using breast cancer and asthma data as examples. Several datamining techniques for clustering of genes and tissues will also be discussed.

The GDB human Genome Database in the post-sequence era. *A.J. Cuticchia¹, C.J. Porter¹, W. Zhu¹, C.C. Talbot Jr.²*. 1) Bioinformatics Supercomputing Centre, The Hospital for Sick Children, Toronto, ON, Canada; 2) Genome Database, The Johns Hopkins School of Medicine, Baltimore, MD.

For the past ten years, the Genome Database (GDB) has provided curated information about genes, mapping reagents, genome maps and genome variations free of charge to the research community. GDB is now integrating its large information base with the human genome draft sequence to provide a complete, well-curated, sequence annotation.

GDB's next release builds upon its greatest strength, peer-reviewed scientific data, while shifting its focus and frame of reference from maps toward the emerging human genome sequence. The database will consolidate multiple sources of reference sequence as its basis for the placement of genes, markers and variations. A GDB query on a gene, region or target sequence will yield an overview of sequence, EST, variation, and relevant mapping data. From there links will provide access to related objects in GDB, and to raw sequence information, annotation and relevant information in other databases worldwide.

As a member of the genome annotation consortium, GDB continues to support the process of community curation and is working with the GDB mirror sites to deploy the annotation software under development by groups at Oak Ridge National Labs and the University of Pennsylvania. GDB will also work closely with HUGO as it develops its genome annotation policies. It is planned that GDB will be the reporting site for gene discoveries peer-reviewed as part of the annotation process.

A Skeletal Gene Database. *N.C. Ho¹, L. Jia¹, C.C. Driscoll², E.M. Gutter³, C.A. Francomano¹*. 1) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Department of Integrative Biology, University of California at Berkeley, Berkeley, CA; 3) Department of Biological Sciences, Ohio University, Athens, OH.

Organized documented data coupled with ready accessibility are of great value to research in human development and disease. The rapid advancement of innovative genomic technology together with the explosion of knowledge witnessed in recent decades on gene and protein sequence, structure, organization, position, expression and function, call for establishment of catalogs and databases to afford rapid referencing. Each database presently in existence covers a myriad of topics as well as furnishing links to related databases, facilitating swift and efficient retrieval of information electronically. The primary objective of Skeletal Gene Database is to create a contemporary list of skeletal-related genes, offering the following information for each gene: gene name, protein name, cellular function, disease(s) caused by mutation of the corresponding gene, chromosomal location, LocusLink number, gene size, exon/intron numbers, mRNA coding region size, protein size/molecular weight, OMIM number of the gene, UniGene assignment and PubMed reference. The Skeletal Gene Database is designed in three phases. The first phase involves cataloguing known skeletal-related genes, the mutations of which are causally associated with human and murine diseases. The second step concentrates on genes that are already documented in the literature and are known to have a major role in the physiological process of skeletogenesis but are not, at this point of time, known to be associated with diseases. The third stage will focus on newly-discovered novel genes expressed in skeletal-related tissues. It will be available on the web in 2000. The data provided will be succinct and current and will continue to be updated as new information comes through. This project will contribute toward understanding the functional genome of bone and bone-related tissues and will be a valuable aid and useful search tool to scientists and physicians interested in topics and research on the skeleton.

GENATLAS: A database for annotation of genes and disease phenotypes in human. *C. Penet, M.L. Chauvet, C. Mugnier, A. Munnich, J. Frezal.* Rene Descartes University, Hosp Necker, Paris.

Funded in 1986, the GENATLAS database was originally aimed to the goal of registering mapped genes in human. GENATLAS has gradually evolved and has become a database that endeavors to link and integrate the informations regarding genetic diseases to the relevant disease causing genes. GENATLAS currently compiles 12,000 genes and 2,000 phenotypes, including 1,400 Mendelian disorders. Available informations include i) physical and genetic mapping data, ii) expression patterns and characteristics of gene transcripts and isoforms, iii) intra- extracellular locations of the gene products, iv) domains, motives and associations or integrations of gene products to complex structures (eg: cell cycle, metabolic or transduction pathways) and v) biological functions (eg: the reaction catalyzed by an enzyme) and categories of protein to which the gene products belong (eg: receptor, transcription factor). GENATLAS puts particular emphasis on the description of disease phenotypes, including Mendelian disorders, developmental anomalies related to contiguous genes syndromes and microdeletions, common diseases and susceptibility to malignancies with particular attention to specific chromosomal rearrangements. Easy links to relevant databases including OMIM, LDB, Locuslink and mutations and sequences databases are also available. The current worldwide sequencing efforts make annotation of the human genome for disease genes and disease phenotypes particularly challenging and rewarding. For these reasons, we believe that GENATLAS should help achieving the annotation of the human genome with respect to disease genes and disease phenotypes. GENATLAS website: <http://www.infobiogen.fr>.

Gene Nomenclature: An Essential Resource. *H.M Wain, E.A Bruford, R. Lovering, M.S Povey.* The Galton Laboratory, Department of Biology, University College London, Wolfson House, 4, Stephenson Way, London, NW1 2HE, UK.

It's time to talk about gene nomenclature. People need and like symbols for genes, and though it may be more logical to give each gene a reference number, people like to personalise and label things. Standardised gene nomenclature is an essential resource for all scientists and the Human Gene Nomenclature Committee (HGNC) is committed to supplying unique gene symbols and names for the ever expanding number of identified human genes. Designations describing structure, function or homology are preferred, where possible, and we work closely with researchers in the field, other online databases including LocusLink, and the Mouse Nomenclature Committee. We have recently been working on the newly finished sequences for chromosomes 22 and 21 (Dunham et al. 1999; Hattori et al. 2000), providing a total of 575 approved symbols. Indeed, for the chromosome 21 publication we were able to ensure that 88% of the genes (including pseudogenes) had approved symbols. With the ever-increasing use of electronic databases this will greatly aid future information retrieval for all interested in the human genome. We are now working with other chromosome-specific databases to provide further designations; so NOW is definitely the right time to talk Nomenclature. The Human Gene Nomenclature Committee webpage can be found at URL <http://www.gene.ucl.ac.uk/nomenclature/> and we can be contacted via nome@galton.ucl.ac.uk.

The pericentromere of chromosome 22q11.2: the birthplace of two novel transcription units formed via the duplication of fragments from various chromosomes. *L.J. Bridgland, T. Footz, A. Riazi, H.E. McDermid.* Biological Sciences, University of Alberta, Edmonton, Alberta, Canada.

Centromeres and the regions around them have not been well studied since they are extremely difficult to sequence and were believed to be gene-poor. Recent evidence indicates that the pericentromeric regions of human chromosomes are preferential sites for the integration of duplicated DNA fragments. The pericentromeric regions of many chromosomes have been examined and at first glance they appear to be junkyards for genic fragments and incomplete genes. However, it has been suggested that the pericentromeric DNA may also be the birthplace of new genes with novel functions (Jackson et al., 1999; Horvath et al, 2000). To date our lab has identified 14 putative genes located in the second megabase from the centromere on human chromosome 22q11.2 as part of a study on cat eye syndrome. The proximal end of this region (the pericentromeric DNA) is extremely gene-poor and is a patchwork of sequences duplicated from every other human chromosome, with very little of the sequence being unique to chromosome 22. Although most of the genes in the region are non-functional and incomplete, at least two genes are expressed from chromosome 22. CECR7 (cat eye syndrome critical region gene 7) is comprised of fragments that show high identity to sequence on chromosomes 2, 7, 10, 11, 12, 13, 15, 16, 18, 21 and elsewhere on 22. These pieces of DNA from across the human genome have been duplicated to numerous pericentromeres, including the pericentromere of chromosome 22q11.2 where they have integrated and been spliced together to form a novel multi-exon transcript. CECR8 is highly similar to sequence on chromosome 8 and is 89% identical to a Y-linked zinc finger protein gene. CECR8 appears to be transcribed in a testis-specific manner. These two putative genes, CECR7 and CECR8, retain significant characteristics of their evolutionary origins as they have arisen by recent duplication events. The study of these putative genes will provide a unique opportunity to better understand the mechanism by which genes originate and how the genome evolves.

Evolutionary conservation of chromosome 21 genes. *K. Gardiner, D. Slavov, A. Fortna.* Eleanor Roosevelt Inst, Denver, CO.

Analysis of the recently completed sequence of human chromosome 21 identified 225 genes and gene models. Functional analysis is now the critical issue for selecting genes of potential relevance to Down syndrome and other chromosome 21 diseases. As a preliminary categorization, protein sequences 122 of the 225 genes/models showed similarities to complete proteins or to protein domains of experimentally verified function, and could be divided among 19 broad functional categories. Many of these similarities are marginally informative, e.g. the presence of a transmembrane domain, an RNA binding domain, or simply an unusual amino acid composition. For more than 30 complete cDNAs (and most gene models), there were no identifiable functional domains or motifs, and therefore no clues as to function. For these, and even for many proteins with suggested functions, using the genetics of model organisms can be expected to be insightful and it is important to catalogue homologous genes. The essentially complete protein database for *Drosophila* was therefore used in a comparison with chromosome 21 proteins. For the ~130 genes with complete protein sequences, 30 showed significant similarity throughout the entire protein to a *Drosophila* protein and 34 showed significant similarities restricted to domains. Of these, 14 proteins were of unknown function in human. Interestingly, this suggests that the remaining novel genes are vertebrate specific. Protein similarities were typically 50%-60%, but ranged as high as 75%-80%, extending over an average of 300 amino acids, up to 1500. Similar analyses were carried out using the protein databases of *S. cerevisiae* and *C. elegans*, and the protein and EST databases for mouse and zebrafish. Patterns of evolutionary conservation can help to identify domains of functional importance, especially in novel proteins. These can then be analyzed through the genetic manipulation and the growing mutational databases of model organisms.

Neocentromere emergence in evolution. *M. Rocchi, M. Ventura, N. Archidiacono.* DAPEG - Sezione Di Genetica, Universita' Di Bari, Bari, Italy.

Evolutionary centromere repositioning is a paradox we have recently discovered while studying the conservation of the phylogenetic chromosome IX in primates (Gen. Res. 9: 1184-1188, 1999). Two explanations were proposed: a conservative hypothesis assuming sequential pericentric inversions or a more challenging assumption involving neocentromere emergence during evolution. The complex evolutionary history exhibited by chromosome IX did not allow us to clearly distinguishing between these two hypotheses. We report here comparative studies on marker order conservation among the X chromosome of HSA and two Lemuridae species: *Eulemur macaco* (Black lemur) and *Lemur catta* (Ring-tailed lemur). They were selected for this study because the morphology of their X chromosome is quite different from that of humans. In spite of the striking morphological differences our results have indicated that marker order is perfectly conserved, unequivocally pointing to the neocentromere emergence as the most likely explanation of the centromere repositioning paradox. Neocentromeres have been documented only in human clinical cases. Our data indicate that they played a role in genome evolution.

A Primary Transcript Map for the Familial Juvenile Hyperuricemic Nephropathy (FJHN) Critical Region on Chromosome 16p11.2. *S. Kmoch*¹, *B. Stiburková*¹, *L. Ondrová*¹, *M. Zikánová*¹, *I. Sebesta*³, *J. Majewski*², *J. Ott*². 1) Inst Inherited Metabolic Dis, Charles Univ 1st Sch Medicine, Prague 2, Czech Republic; 2) Laboratory of Statistical Genetics, Rockefeller University, New York; 3) Department of Clinical Biochemistry, 1st School of Medicine and General Faculty Hospital Prague.

Familial juvenile hyperuricaemic nephropathy (FJHN) is an autosomal dominant renal disease, characterized by juvenile onset of hyperuricemia, gouty arthritis and progressive renal failure at an early age. Using a genomewide linkage analysis in three Czech affected families we have identified a locus for FJHN on chromosome 16p11.2 and found evidence for genetic heterogeneity and reduced penetrance of the disease (Stiburková et al, (2000) Am J Hum Genet 66:1989-1994). Haplotype analysis defined a 10 cM candidate region between flanking markers D16S501 and D16S3113 exhibiting crossover events with the disease locus. Our results and a recent publication on localization of the FJHN gene in another family (Kamatani et al. (2000) Arthritis Rheum 43: 925-9) have narrowed the 1.7 cM candidate region, between markers D16S403 and D16S3113. The entire FJHN candidate region is covered by a BAC clone contig (Caltech). 13 overlapping clones spanning approximately 2 Mb were selected for further analysis. The complete genomic sequences available for 8 of the clones were analyzed using BLAST homology searches against various EST and protein databases (GenBank, HGI, STACK) and various gene prediction programs (Genescan, Grail) in order to establish a transcriptional map and identify putative exons. So far, these analyses revealed the presence of 35 putative transcripts. Expression profiles of those transcripts are analyzed in-silico (HGI, Unigene) and individual transcript cDNAs are subsequently prepared using PCR-RACE. The full-length cDNAs are cloned, sequenced and its expression profiles are studied on a multiple tissue cDNA panel. Genes expressed in the kidney are prioritized for mutation analysis in affected pedigrees.

Human BAC Clones: From Chromosomes To Sequences. *V.G. Cheung, S. Narasimhan, M. Arcaro, T. Weber, J. Burdick, M. Morley, A. Bruzel.* University of Pennsylvania, The Children's Hospital of Philadelphia, Dept of Pediatrics, Philadelphia, PA 19104, USA.

We are mapping, fingerprinting and end-sequencing a set of human BAC clones that span the human genome at about 1-Mb intervals. The clones are designed as reagents for cytogenetic analyses and for microarray-based gene mapping and chromosomal mutation detection studies.

The BAC clones are part of the Roswell Park Cancer Institute human male BAC library (RPCI-11). The clones are mapped to sequence-tagged site (STS) markers by filter hybridization and PCR. In addition, their locations and orderings are confirmed by fluorescent in situ hybridization. The clones are characterized by *HindIII* fingerprinting and end-sequencing. The position and sequence information of the clones serve as means to integrate chromosomal locations, STS map and human genomic sequences. Using the clones as probes in cytogenetic analyses or microarray-based studies, one can navigate from chromosomal bands directly to DNA sequences. All the information about the clones is available on our database, GenMapDB, <http://genomics.med.upenn.edu/genmapdb>. To date, we have information for clones on chromosomes 2, 4, 5, 9-22, X and Y. In this presentation, we will describe the progress of this mapping project and will illustrate how this resource can be used in genetic analyses.

FISH on extended chromatin and PFGE: a complementing approach for high resolution mapping of deletion breakpoints in NF1 patients. *P. Riva, A. Bentivegna, C. Gervasini, L. Corrado, M. Venturin, F. Natacci, L. Larizza.* Dept Biol & Genetics, Univ Milan, Italy.

Large NF1 deletions arise preferentially on maternal chromosomes, suggesting that the underlying molecular mechanism is recombination amplicon mediated in 17q11.2. Evidence of low-copy-repeat (LCRs) flanking deleted regions is achieved in an increasing number of microdeletion syndromes. By performing high resolution FISH of gross deletions from nine NF1 patients, we found that their proximal breakpoints cluster to the same region. In order to verify the presence of LCRs we addressed the precise mapping of a large deletion in one NF1 patient, for whom a lymphoblastoid cell line was available, by PFGE and FISH on extended chromatin. FISH with probes specific for D17S975 D17S1317 D17S1294 showed that D17S1317 and D17S975 are flanking the deletion, while D17S1294 is deleted. On the basis of the reciprocal anchorage of the three markers on a 17q11.2 YAC contig, we established that the first proximal locus mapped at the centromeric breakpoint is D17S1317, which is expected to be located between D17S975 and D17S1294, while the telomeric boundary of the deletion was fixed to MCP3 gene. We then selected D17S1317 and MCP3 specific PAC clones from a RCPI-PAC library to evaluate the relative breakpoint distances from the two anchored markers on the deleted chromosome. Double color FISH experiments at different resolution degrees were performed on metaphases and stretched chromosomes and on chromatin fiber with the two selected PACs. We observed on one stretched chromosome 17 D17S1317 and MCP-3 specific signals separated by a chromatin segment while on the homologue the two signals merged, indicating that the distance between these loci on the deleted chromosome is <200 Kb. An independent evidence for this finding was provided by detection of a novel DNA fragment by PFGE hybridization with an MCP3 specific probe. The double color FISH on chromatin fiber allowed to observe that the two PACs are not only contiguous, but partially overlapping, suggesting that LCRs might be present and mediate the homologous recombination leading to microdeletion. Supported by Telethon Grant E780.

Novel ancient sequence genes on human chromosomes 22 and 21. *G.A. Bruns^{1,2}, R.E. Eisenman¹*. 1) Genetics Division, Children's Hosp, Boston, MA; 2) Dept of Pediatrics, Harvard Medical School, Boston, MA.

By cross-species database searches, we have identified a number of human genes that have significant homology in the *D.melanogaster* and/or *C.elegans* genomes but do not recognize known proteins, protein families or motifs. These genes are members of a small subset of human loci that encode previously unknown ancient conserved sequences. They are index members of new gene families and correspond to some of the undiscovered ancient sequence loci predicted to exist by Green et al (Science 259:1711-1716,1993). Ancient conserved sequence regions (ACRs) are areas of proteins with fundamental functional or architectural significance and often are the "class-marking" domains that define categories of proteins. Mutations of genes encoding ACR proteins are frequently associated with developmental disorders.

We have identified 10 novel ancient sequence genes on chromosome 22. BLAST searches of the *C.elegans* Wormpep 18 database, the *D.melanogaster* EST database, and the *D.melanogaster* genomic sequence were done using chromosome 22 ESTs defined on the genomic sequence that themselves identified no known protein or motif. Any retrieve that identified a homolog of known function or a member of a function family was excluded, as were those that encoded a recognizable motif. The E values for 9 of the genes ranged from 3×10^{-18} to 0.0. For the 10th, where the homology likely represents a short functional motif, the E value was 5×10^{-5} . Eight of the chromosome 22 genes identified homologs in both the *C.elegans* and *D.melanogaster* genomes; one recognized a homolog in *C.elegans* alone; and one, only in *D.melanogaster*. For 6 of the genes, the areas of cross-species similarity extended throughout the protein, a feature observed with highly conserved structural proteins and certain catalytic molecules. These "ancient sequence" genes, with known map positions, are candidate loci for human inherited disorders involving chromosome 22. The set of novel ancient sequence genes on chromosome 21 will be similarly identified.

Human biotin-containing subunit of 3-methylcrotonyl-CoA carboxylase gene (MCC-2): cDNA sequence, genomic organization, and localization to chromosome 3q27. *T. Fukuda*¹, *K. Obata*¹, *R. Morishita*¹, *K. Shigemoto*¹, *S. Abe*², *M. Yoshino*³, *S. Yamaguchi*⁴, *S. Asakawa*⁵, *N. Shimizu*⁵, *I. Kondo*¹. 1) Dept. of Hygiene, Ehime Uni. School of Med., Ehime, Japan; 2) Dept. of Biological Resources, Faculty of Agriculture, Ehime Univ., Ehime, Japan; 3) Dept. of Pediatr. and Child Health, Kurume Univ. School of Med., Fukuoka, Japan; 4) Dept. of Pediatr., Shimane Med. Univ., Izumo, Japan; 5) Dept. of Mol. Biology, Keio Univ. School of Med., Tokyo, Japan.

3-methylcrotonyl-CoA carboxylase (MCCase, EC 6.4.1.4) is a mitochondrial biotin enzyme, and plays an essential role in the catabolism of leucine and isovalerate in animals, bacterial species and plants. MCCase consists of two subunits: biotin-containing and non-biotin-containing subunits. The gene responsible for these subunits has been isolated in *Arabidopsis thaliana* but not in mammals. In human, MCCase deficiency appeared increasing with a wide range of clinical presentations; some that result in lethal conditions, and others that are non-symptomatic. We have isolated and carried out chromosomal mapping of the gene for the biotin-containing subunit (beta-subunit) of the human MCCase, *MCC-2*. *MCC-2* cDNA contained an open reading frame encoding for a 725 amino acid protein with mitochondrial signal peptide, biotin carboxylase and biotin-carrier domains. The gene is composed of at least 19 exons and covers more than 70kb of sequence on q27 of chromosome 3. *MCC-2* was abundantly expressed in mitochondrial-rich organs, such as the heart, skeletal muscles, kidney and liver. In exon 13, a His/Pro polymorphism in codon 464 (an A to C transversion at nucleotide position 1391 in the cDNA sequence) was detected. Then, we determined the DNA sequences of the entire coding regions in two patients with MCCase deficiency, but no sequence changes were detected, suggesting that the gene mutations might be in the non-biotin-containing subunit, MCC-alpha, in these patients.

Mapping of KPNA1, KPNA4 and KPNB1 exclude these genes as candidates for Russell-Silver syndrome. *M.L. Ayala-Madrigal*^{1,2}, *S. Doerr*¹, *M.L. Ramirez-Dueñas*², *I. Hansmann*¹. 1) Inst. f. Humangenetik, Halle, Germany; 2) UdeG and CIBO, IMSS Guadalajara, Mexico.

Members of the karyopherin gene families encode proteins that are involved in the nuclear import of proteins with a nuclear localization signal (NLS). As part of our effort to isolate candidate genes for the heterogeneous Russell-Silver syndrome (RSS) we identified Karyopherin alpha 2 (KPNA2) a member of the karyopherin alpha family in close proximity to a disease associated translocation breakpoint within 17q23-q24. Furthermore, we found a specific haplotype comprising 6 intragenic polymorphisms to be associated with RSS. Therefore, KPNA2 is a positional candidate gene for RSS. This mostly sporadic disorder is mainly characterized by pre- and postnatal growth retardation and characteristic dysmorphic features. Because protein import is necessary for the function of hormones and transcription factors involved in growth regulation, KPNA2 is a good candidate gene for the disease. Since KPNA2 is part of a multigene family and RSS is a heterogeneous disorder we supposed that if KPNA2 plays a role in generating the defects typical for RSS other genes of this family may also be involved in the etiology of RSS. Therefore, we isolated PAC clones for the so far unmapped genes KPNA1, KKPNA4 and KPNB1. These clones were used for FISH analysis and we were able to localize these genes to chromosome 3q21, 11q22 and 17q21, respectively. Several chromosomal abnormalities have been reported in patients with features suggestive for RSS. These abnormalities include partial duplications of 1q, duplications/inversions of 7p, maternal uniparental disomy of chromosome 7, deletions within 8q, deletions of distal 15q or ring chromosome 15, deletions within chromosome 18p, and translocations involving chromosome 17q. So far no chromosomal abnormalities within 3q21, 11q22, and 17q21 were reported in patients with RSS. Therefore, it is unlikely that the three genes localized to this regions play a role in the etiology of RSS. Mapping of the genes could be an important step to enlight the cause of other disorders.

Physical mapping and further refinement of Athabascan Severe Combined Immunodeficiency Disease region. *Y. Zhou, L. Li, M. Cowan.* Department of Pediatrics, UCSF, San Francisco, CA.

Athabascan Severe Combined Immunodeficiency Disease (SCIDA) is a distinct form of SCID found in three Athabascan-speaking Native American tribes. SCIDA is associated with prominent defect of both T and B cell immunity. We have previously mapped the disease gene to a 6.5cM interval on chromosome 10p by linkage analysis and narrowed the critical region to 2 cM by subsequent linkage disequilibrium and haplotype analyses. Also, the data showed that SCIDA is linked to a founder effect. In order to further narrow the disease region and identify putative candidate genes for SCIDA, we constructed a YAC contig and a BAC/PAC contig covering the 2cM critical interval and physically mapped the disease region. The YAC and BAC/PAC contigs allowed us to accurately map 2 genes (RPP38 and NMT2) and 5 ESTs previously mapped to the disease region or nearby interval. We have excluded the two genes as SCIDA candidate genes and the study for the 5 ESTs is undergoing. We generated over 30 new STRP markers from the BAC/PAC contig and genotyped these markers on 26 SCIDA patients and 30 normal controls. Further linkage disequilibrium and haplotype analyses with these new markers allowed us to narrow the SCIDA critical region to a ~500kb interval. A combined positional cloning effort is undertaken to identify and study all the candidate genes within this region.

Transcript map of Athabascan Severe Combined Immunodeficiency Disease gene locus on chromosome 10p13. J. Wang, L. Li, Y. Zhou, M. Cowan. Department of Pediatrics, UCSF, San Francisco, CA.

Severe Combined Immunodeficiency Disease with T-B- phenotype (T-B-SCID) is inherited as an autosomal recessive trait. There is a uniquely high frequency of T-B-SCID among Athabascan-speaking Native Americans (SCIDA). Genetic mapping has assigned the SCIDA locus to a region of approximately 2Mb on chromosome 10p13. To facilitate the disease gene identification, a physical map of this region has been generated using bacterial artificial chromosome (BAC) and P1 artificial chromosome (PAC) clones. A combination of CpG island identification, direct cDNA selection, exon trapping and the analysis of DNA sequence in public database have been used to generate a transcript map for SCIDA critical region. Fine mapping has localized 28 novel transcript units including exons, cDNAs and cDNA contigs besides several cDNA fragments representing 2 known genes (NMT2 and RPP38) and 2 pseudo-genes to the region of interest. Analysis of the expression patterns and transcript size of these novel transcripts as well as full-length construction and mutation screening are currently ongoing.

Regional Mapping Panels for Human Chromosomes 10 and 12. *L.H. Toji, J.C. Leonard, P.K. Bender, C.M. Beiswanger, J.C. Beck, R.T. Johnson.* Coriell Cell Repositories, Coriell Inst Medical Research, Camden, NJ.

The NIGMS Human Genetic Cell Repository is establishing a collection of well-characterized somatic cell hybrid regional mapping panels for each human chromosome. With the cooperation of its advisers and various chromosome committee members, the CCR has identified regional mapping panels for chromosomes 10 and 12 from the hybrids in the collection submitted by D. Callen, B. Emanuel, R. Gemmill, K. Grzeschik, D. Ledbetter, D. Patterson, T. Shows and J. Wasmuth. These panels define 8-9 intervals for each chromosome. The submitters characterized the hybrids originally; analyses performed at the CCR supported the submitters' descriptions of the hybrids. The human chromosome content of the hybrids was confirmed by G-banding and in situ hybridization with total human DNA and in some cases, with specific chromosome paints or alpha satellite probes and by Comparative Genomic Hybridization (CGH). In addition, DNA samples prepared from the hybrids were characterized by Southern blot hybridization and/or by PCR with probes or primers for each human chromosome in the specific human derivative or deletion chromosome. The NIGMS Human Genetic Mutant Cell Repository now has regional mapping panels for chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 21, 22 and X. These panels are available as cell cultures or as DNA. Information about these hybrids may be viewed in the NIGMS Repository electronic catalog (<http://locus.umdj.edu/nigms>).

Whole Genome Amplification of Centenarian DNA: A Means of Providing DNA from Limiting Tissue Sources.

P.K. Bender, M. Schmidt, J.C. Beck, R.T. Johnson. Coriell Cell Repositories, Coriell Inst Medical Research, Camden, NJ.

Recently, the National Institute of Aging Cell Repository received a posthumous biopsy sample from a 115-year-old donor. Only a small amount of material was received, and it had been frozen at -20C in sub-optimal conditions for cell line development. All attempts to establish cultures from this material failed. This tissue thus became an obligate candidate for whole genome amplification (WGA) to preserve its genetic information and provide sufficient DNA material for distribution to the research community. The WGA method selected by the CCR is based on a technique called primer-enhanced-preamplification (PEP). PEP relies on fully degenerate primers to target the PCR amplification of sequences throughout the genome without bias for sequence content. A sliver of centenarian skin tissue, approximately 2 X 5 mm, was processed through proteinase K digestion and organic extraction to purify the genomic DNA. The yield of DNA was approximately 10 mg. From this material, twenty 1.0 ng DNA aliquots were subjected to PEP, and the resulting PEP products pooled into 2 lots of 10 reactions each. Measurements of DNA amounts revealed a 2,000-fold amplification of the genomic DNA template and a size of the amplified DNA ranging from 500-6,000 bp. Several molecular assays were undertaken to determine the allelic bias in the samples and assess the usefulness of the material for genetic analysis. Presence of the X- and Y-chromosome amelogenin alleles was measured and both alleles were found to be present, confirming the gender of the initial sample. Allelic representation of 5 microsatellites was determined in the PEP material and gave the same genotype as that of the starting material. Assay of the PEP material for its ApoE genotype by RFLP analysis was successful and shows it to be an e2/e3 heterozygote. These data on a 115-year-old individual's tissue indicate that WGA generates DNA that is representative of the original genomic material at many loci. These results provide a framework for development of a centenarian DNA repository that combines cell culture and, when necessary, WGA to generate sufficient DNA for distribution.

Implementation of an Industrial Process for High-Throughput Genotyping of Single Nucleotide Polymorphisms by Single-Base Primer Extension. *F. Watson, L. Picoult-Newberg, M. Pohl, M. Phillips, D. Grant, M. Boyce-Jacino.* Orchid BioSciences Inc., Princeton, NJ.

The completion of the Human Genome Project heralds the advent of the next crucial phase in the genomics revolution -- the discovery, confirmation, functional characterization and clinical correlation of the millions of genetic variations that exist within the human genome. To enable studies in functional and correlative genomics to proceed at the pace required by both the flood of available template material and the needs of the pharmaceutical industry for new drug targets, technologies for the genotyping of single nucleotide polymorphisms (SNPs) at high throughputs are required. Orchid BioSciences has developed a proprietary SNP genotyping technology (SNP-IT™ SNP Identification Technology) based on single-base primer extension. In this method, a single-stranded PCR amplicon containing the SNP of interest is hybridized to an immobilized SNP-IT capture oligonucleotide, which terminates immediately adjacent to the SNP. Addition of DNA polymerase and labeled dideoxynucleotide chain-terminating bases leads to the incorporation of a labeled base at the site of the SNP, whose identity can be determined by a variety of detection methods including optical density, fluorescence or mass spectrometry. SNP-IT possesses key features for adaptation to automated platforms, including specificity, accuracy, reliability, ease of use, reproducibility, low cost and flexibility. Another key requirement for industrialized SNP scoring is efficient assay design. We have developed assay formatting algorithms for the development of genotyping assays with a high frequency of first-pass success. The SNPstream system processes samples in a 384-well plate format, with 370 genotypes per plate. A Beckman/Sagian robotic backbone enables the instrument to process batches of 10 or 20 plates per day, yielding a 24 hour capacity of more than 20,000 genotypes. The system can be used for SNP validation or for population analysis in gene mapping or association studies. Our facility currently utilizes 4 SNPstreams and has developed genotyping assays for several thousand SNPs and generated several million genotypes.

Nearest-neighbor DTm analysis of 102,900 SNPs predicts general applicability of 5' nuclease propyne T probes.

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The 5' nuclease method of SNP scoring exploits the difference in melting temperature (DTm) between a probe hybridized with its perfect match complement and the same probe hybridized with a mismatch complement. To date, the general applicability of the 5' nuclease method has been limited due to the hit or miss problem of designing probes with sufficient DTm to achieve robust allelic discrimination. Thermodynamic values for all possible single base mismatches are available that allow the nearest neighbor model to be applied to SNPs to predict probe DTm. A computer program that maximizes probe DTm has been implemented and applied to 102,900 putative SNPs (NCBI's dbSNP, release May 19 2000). The constraints were probes with perfect match Tm between 68° C and 72° C, that have the variable position in the middle third of the probe, that do not begin with a G, and have length between 15 bp and 35 bp. Where ND indicates no qualifying probe set, the table shows that 67% of SNPs yielded predicted DTms greater than 3.0° C, a threshold over which probes often yield discrimination. Replacement of the probe T bases with propyne T derivative is predicted to increase Tm 1.0° C for every T in the probe. Propyne T probes have been used successfully in AT rich regions. Applying propyne T probes for SNPs with flanking GC%£60.0 shortened the average probe from 25.0 bp to 21.5 bp, and increased the average DTm 31% from 3.9° C to 5.1° C. These results suggest that probe sets with maximal DTm can be predicted, and SNPs with DTm less than 3.0° C can be avoided. Further, propyne T probes predict success of the 5' nuclease method for all but a small fraction of SNPs.

DTm	ND	1	2	3	4	5	6	7
RegularT	17%	2%	14%	29%	22%	11%	4%	1%
PropyneT	0%	0%	1%	10%	32%	40%	15%	2%

Sequence Analysis of Human Chromosome 15q11-q14: An Interval Associated with Multiple Genomic Disorders.

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Employing emerging rough draft sequence for the human genome, we have integrated, assembled and analyzed sequence for the region in proximal 15q corresponding to interstitial deletions observed in Prader-Willi and Angelman syndromes, and interstitial and inverted duplications and triplications associated with autism-spectrum phenotypes. These genomic disorders are caused by mispairing and subsequent recombination between homologous, low-copy repeated sequences present at multiple breakpoints, or so-called duplicons, in 15q11-q14. Duplication or deletion generates increased or decreased gene copy and expression for the affected intervals, further complicated by genomic imprinting. Specific genes underlying the Prader-Willi and autistic phenotypes remain unknown but lie within intervals defined by different deletion and duplication breakpoints. To apply emerging human genome sequence to this chromosomal region, known genes, STS/EST markers and other existing genomic sequences were utilized as queries in an iterative process to identify genomic sequence in the HTGS database. A contig spanning the entire interval with few intermittent gaps was developed. Contiguous sequence across the proximal 15q region is resolving marker order and position, allowing for accurate determination of physical distances between genes and other landmarks, and integrating genetic and physical maps. Genomic sequence is facilitating the discovery of putative new genes, is providing gene structure information for uncharacterized genes, and will promote identification and analysis of regulatory elements for genes in this region. In addition to the use of overlapping sequences to facilitate identification of single nucleotide polymorphisms (SNPs), availability of the primary sequence will permit re-sequencing for SNP discovery; this is critical for analysis of complex disorders such as autism. Finally, genomic sequence is aiding in the identification and characterization of chromosomal breakpoints and the mechanisms underlying the formation of various chromosomal abnormalities involving proximal 15q.

Systematic gene identification based on the genomic sequence of human chromosome 21. *J. Kudoh¹, K. Shibuya¹, H.S. Scott², K. Nagamine^{1,3}, K. Kawasaki¹, A. Shintani¹, T. Sasaki¹, J. Wang¹, M. Tatsuyama¹, M. Guipponi², J. Michaud², L. Bartoloni², M. Wattenhofer², C. Rossier², S. Mitsuyama¹, S. Asakawa¹, S. Minoshima¹, S.E. Antonarakis², N. Shimizu¹.* 1) Department of Molecular Biology, Keio University School of Medicine, Tokyo, Japan; 2) Division of Medical Genetics, Geneva University Medical School and University Hospitals, Geneva, Switzerland; 3) Eiken, Tochigi, Japan.

We have finished genomic sequencing of chromosome 21q in collaboration with RIKEN GSC (Japan) and German sequencing centers (IMB, GBF and MPIMG) and have identified 127 known genes, at least 98 predicted genes and 59 pseudogenes in the 33.5-Mb genomic sequence (Nature 405:311-319, 2000). We have determined 6.5 Mb (about 20% of 21q) of sequence with estimated accuracy of >99.999%. Genomic sequence was subjected to homology search and analysis for protein coding potential using GRAIL, MZEF, and GENSCAN. Putative exon sequences were used to amplify cDNA fragments by PCR or to screen corresponding cDNA clones from various human tissues. Nucleotide sequences of these cDNA fragments and those registered in databases allowed us to identify 45 new genes including C21orf5, DSCR5, DSCR6, ZNF295, UMODL1, TMPRSS3, UBASH3A, TSGA2, SLC37A1, PDE9A, WDR4, SNF1LK, H2BSF, AGPAT3, TRPC7, AIRE, DNMT3L (collaboration with P. Peterson and K. Krohn), and a cluster of keratin-associated protein (KAP) genes in which putative genes are repeated 18 times in a 164-kb region, 24 known genes including ADAMTS1, ADAMTS5, and HSF2BP, and 15 pseudogenes including six ribosomal protein-related. Among these, TMPRSS3 encoding a putative transmembrane serine protease was found to be responsible for the autosomal recessive nonsyndromic deafness DFNB10 (collaboration with A. Berry and B. Bonne-Tamir). The transcript map and sequence information are available through our Web site (<http://www.dmb.med.keio.ac.jp>) which should be useful for further finding of new genes, identification of disease related genes, and prediction of their function.

An independent evaluation of the current status of the Human Genome. *N. Katsanis, K.C. Worley, J.R. Lupski.*
Baylor College of Medicine, Houston, TX.

A draft version of the human genome encompassing 90% or more of euchromatin will shortly become available. The value of this information is obvious, but the extent of genome coverage, mapping accuracy of individual sequences and contiguity of sequenced segments remains unclear. To evaluate these issues we have performed a series of analyses using 925 ESTs collected during our efforts to identify novel human retinal transcripts and the corresponding draft genome sequence. These analyses were performed on three different versions of genomic sequence: draft sequence at the 75% completion level (April 15 GenBank version), draft sequence at the 90% completion level (final version) and finished sequence (~20%, June 15 GenBank version). To examine the percentile coverage, we performed BLAST searches of all 925 ESTs and identified matches with genomic sequence using highly stringent criteria (>98% similarity over the entire EST sequence and > 99% similarity over the first 200 bp of the EST sequence). The percentage of ESTs positive for genomic sequence is a true representation of the coverage level of the draft sequence, which was found to be ~12% below reported figures. The second test addressed the percentile redundancy in the draft sequence by counting the number of BACs matching each EST. This analysis revealed redundancy levels as high as 47%, a figure once again higher than expected. The final test correlated the mapping position of BACs, as inferred from their annotation or electronic PCR, where the entire BAC sequence is used to search for known STSs, with the mapping position of ESTs ascertained by monochromosomal and radiation hybrid mapping. We identified substantial discrepancies in BAC annotation with regard to chromosomal assignment. Of particular note was the discrepancy rate in the draft sequence (25-33%). Finished sequence discrepancies were much lower (~7%). We conclude that there are substantial differences between the reported and the actual status of the draft sequence. The latter is of particular importance since it suggests that although the draft genome sequence is a powerful tool for whole-genome analyses, such as identifying novel gene family members, it is less useful where positional information is required.

Semi-Automated Oligo Ligation Assay (OLA) with ABI3700 Sequencer Allele Resolution and Complete Sample Tracking. *R. Rallan, L.C. McCarthy, D.S. Montgomery, D.G. Doreen, K.L. Cantone, C. Christodoulou, R.J. Cutts, C. Donnelly, R.W. Gill, A-S. Merot, M.D. Oxe, P. Robinson, S. Shaw-Hawkins, T. Tavinder, R.W. Tothill, I.J. Purvis.* Molecular Genetics, Glaxowellcome, Stevenage, Herts, UK.

The rapid increase in numbers of SNPs in the public domain has necessitated the development of high-throughput cost effective genotyping techniques. By combining multiplex PCR with multiplex oligo ligation assay (OLA), a substantial saving can be made on reagents, consumables, time and labour costs. We have automated the liquid handling steps in this process, and incorporated complete sample tracking with barcodes such that all manipulations performed on each DNA sample can be traced. Run parameters were determined for resolving the SNP alleles on ABI3700 sequencers to increase the throughput of the technique. A pilot study has been completed to compare the results using this method with those obtained using Taqman, our current method of choice for medium throughput genotyping. Advantages of 3700 OLA include DNA efficiency and cost effectiveness. Sub-optimal multiplex PCR provides enough template for multiplex OLA, enabling genotyping with 5% of the DNA required for most other PCR-dependent genotyping techniques. The cost of this method is 0.22 per genotype using multiplex PCR and OLA, making it more cost effective than most available genotyping techniques. Using a single ABI3700 sequencer with 20x multiplexed OLA reactions, up to 25,000 genotypes could be resolved per day.

HUMAN AND MOUSE BAC ENDS. *S. Zhao, M. Adams, J. Malek, L. Fu, B. Akinretoy, S. Shatsman, K. Geer, M. Levins, G. Tsegaye, M. Krol, T. Feldblyum, W. Nierman, C. Fraser.* Fraser The Institute for Genomic Research, Rockville, MD.

Large scale human BAC end sequencing has been conducted at TIGR and UWashingtong using CalTech BAC libraries and RPCI-11. At TIGR, we have generated >300,000 BAC end sequences (BESs) from >186,000 clones. The average reads are ~460 bp. Over 60% of the clones have BESs from both ends. The average phred Q20 length is ~400 bp, allowing the BESs matching the finished sequences with an identity of 99%. The clone tracking accuracy is >90%, giving researchers a high confidence in retrieving the right clones from BAC libraries based on sequence matches. Additional sequencing performed at UW has resulted in >700,000 total BESs. Our quality assessments indicate that BESs from TIGR and UW are sufficiently accurate for use in large-scale sequencing projects. The analyses have highlighted differences in insert size for different segments of the CalTech library. Problems with the fidelity of tracking of sequence data back to physical clones have been observed in some subsets of the overall BES dataset. These results demonstrate that the BES data are not uniform in quality. It is therefore important for users to be aware of this. We have been conducting large scale mouse BAC end sequencing to generate both ends from ~300,000 clones from RPCI-23 and RPCI-24 libraries. Benefited from our human ends experience, we have improved the sequencing protocol and have maintained a success rate of above 80%. To date, we have over 200,000 ends from >113,000 RPCI-23 BACs with 76% of the clones having paired-ends. The average read length is 480 bp and the average Q20 length is 400 bp. The sequencing is conducted with ABI 3700 sequencers and extra tracking checks have been implemented into the process. These insure a tracking accuracy of >98%. We expect that the mouse resource will have an even higher quality than our current human ends. (http://www.tigr.org/tdb/humgen/bac_end_search/bac_end_search.html) (http://www.tigr.org/tdb/bac_ends/mouse/bac_end_intro.html).

Creation of comprehensive protein expression libraries in human cells using genome-wide Random Activation of Gene Expression (RAGE). *B.A. Sherf, S. Rundlett, R. Perry, J. Harrington.* Athersys, Inc, Cleveland, OH.

Random Activation of Gene Expression (RAGE) vectors were used to create genome-wide libraries in a variety of human cell lines. RAGE libraries containing only 5×10^6 individual clones were found to contain clones expressing activated, sequence tagged transcripts for each of 20 genes tested, the majority of which are normally silent in the parent cell line. Furthermore, endogenously expressed genes were activated at similar frequencies to normally silent genes, demonstrating that RAGE libraries are inherently normalized. These attributes of RAGE libraries make them ideally suited for the discovery of novel human genes. Thus far, pools of human cells from RAGE libraries have been used to isolate more than 20,000 individual cDNA's, approximately half of which are found to represent novel genes when tested against public databases. In addition, plasmid, transposon and retroviral versions of RAGE vectors have been specialized to express high levels of the proteins encoded by their activated genes. Genome-wide RAGE protein expression (PE) libraries were constructed and 25,000 member superpools were screened for the expression of a variety of known trans-membrane and secreted proteins. Individual clones expressing the desired targets were subsequently isolated and demonstrated to express high levels of biologically active proteins. RAGE-PE cell lines have been generated to express a number of therapeutic proteins as well as desirable targets for drug discovery. In functional genomics applications, small pools of RAGE-PE library members or their culture supernatants are utilized in high-throughput cellular assays to screen for clones expressing proteins with novel biological activities. Hence, the ability to generate comprehensive protein expression libraries enables the rapid generation of specific cell lines for use in drug development and protein manufacturing, as well as the ability to screen clone pools for the discovery and characterization of novel protein functions.

BioChip SNP analysis assay: Development of a 3-D microarray system. *M.R. Bonner¹, K. McWeeny², P. Gwynne¹, A. Gilbreath¹, J.T. Tuggle¹, T. Peters¹, J. Xia¹, J.D. Winick¹, K. Luehrsen¹, B. Chui¹, M. Gaskin¹, A. Zilbergleyt¹, N. Kroutchinina¹, C.T. Yamashiro¹, T. Kaysser-Kranich², L. Allegri¹, D. Wang², S. Gallagher¹, T.J. Raich¹.* 1) Motorola BioChip Systems, Tempe, AZ; 2) Motorola BioChip Systems, Northbrook, IL.

The analysis of single nucleotide polymorphisms (SNPs) has been demonstrated to have significant value in understanding population structure, in identifying the genetic basis of drug metabolism, and in generating linkage maps for heritable diseases. To address the needs of such projects Motorola BioChip Systems has developed a high throughput SNP analysis system utilizing microarrays on a hydrophilic gel matrix. The analysis makes use of anchored oligonucleotide probes and a polymerase mediated single base extension using PCR amplified DNA target. This system was tested by analyzing a genetically diverse panel of genomic DNAs for 300 autosomal single nucleotide polymorphisms. Call rates and accuracies were calculated based on sequencing information for each individual for each SNP. The BioChip assay system can be easily customized to suit most SNP projects.

ALPHASCREEN™ Homogenous Assays for SNP Genotyping. *L. Beaudet¹, B. Breton¹, J. Bédard¹, M. Budarf².* 1) BioSignal Inc., Montreal, QC, Canada; 2) Division of Human Genetics, Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA.

AlphaScreen homogenous proximity assays were developed to measure various biological interactions. In these assays, signal is generated when a donor and an acceptor bead are brought into proximity. Initially developed for drug discovery applications, the AlphaScreen technology has been adapted to the detection of nucleic acids. In this case, two bridging probes, hybridizing simultaneously to the generic beads and to a common target, are used to bring the beads into proximity. The sensitivity of AlphaScreen for nucleic acid detection was assessed. As little as 20 amol of a single-stranded RNA or DNA can be detected. Plasmid DNA was also detected with a similar sensitivity (100 amol). Addition of genomic DNA to the reactions did not change signal significantly, indicating that complex material does not affect the detection. The dynamic range for nucleic acid quantitation is from 20 amol to 6 fmol. The combination of AlphaScreen with allele-specific amplification (ASA) and allele-specific hybridization (ASH) has allowed the development of two high throughput homogenous genotyping applications. In AlphaScreen ASA, the specificity for allele-detection comes from the 3' end of one of the PCR primers. Twelve ASA assays were developed. A validation study performed in collaboration with academic laboratories showed an accuracy of greater than 99%. Titration of genomic DNA showed that 0.5 ng (in 10 ml reactions) and 30 PCR cycles were sufficient in most assays for accurate genotyping. In ASH, specificity is conferred by the selective hybridization of one of the two bridging probes. ASH assays are also very accurate (validation in progress), with a signal to noise ratio of 15 to 30. The two AlphaScreen genotyping assays have been automated and miniaturized to the 384-well format. Only two additions are required: PCR/AlphaScreen mix and genomic DNA. After PCR and probe hybridization, the 384-well plate is read by the Alpha reader in less than 2.5 min. The high allele-discrimination obtained with both genotyping assays allows the automatic scoring of the genotypes.

Comparison of mRNA kidney expression profiles generated by serial analysis of gene expression (SAGE) to identify candidate nephropathy genes in C57BL/6-Os/+ versus ROP-Os/+ mice. *K.A. Goddard¹, A. El-Meanawy², J.R. Schelling², E. Elashi², S. Barathan², M. Konieczkowski², S. Kamar², A. Koepke², A. Covic², J.R. Sedor², S.K. Iyengar¹.* 1) Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Medicine, Case Western Reserve Univ, MetroHealth Campus, Cleveland, OH.

Novel mechanisms of progressive renal disease can be identified by determining how clusters of gene transcripts coordinately change. To identify genes that regulate renal disease progression, SAGE kidney mRNA profiles were contrasted from sclerosis-prone ROP-Os/+ and sclerosis-resistant C57BL/6-Os/+ (C57-Os/+) mice. Renal function and histology was assessed in ROP-Os/+ and C57-Os/+ mice from 6 to 16 weeks of age to establish the earliest time point of renal disease onset. Glomerular sclerosis score, determined by computer-aided quantitative morphometry, was increased in 6 wk ROP-Os/+ (22.2 [plusminus] 12.8) compared to age-matched C57-Os/+ (15.2 [plusminus] 3.4) mice. Urine albumin/creatinine also was significantly greater in 6 wk ROP-Os/+ mice (8430 [plusminus] 5857) vs age-matched C57-Os/+ mice (1029 [plusminus] 370). Thus far, 2791 and 1713 tags, representing 1737 and 1168 unique genes, have been identified from 6 week ROP-Os/+ and C57-Os/+ expression libraries, respectively. P-values were determined for the difference in tag counts between the two libraries using the winflat program (Genome Research 7:986-995, 1997), assuming a poisson distribution for the number of observed tags. Two tags, which identified an EST with no known function and glutathione peroxidase 3 (Gpx3), respectively, were differentially expressed at a significance level of $p \leq 0.01$. Both tags were more abundant in the sclerosis-prone ROP-Os/+ kidney. Enhanced expression of Gpx3 suggests a compensatory response to increased oxidant stress, which may be an early mechanism of sclerosis pathogenesis in this model. We conclude that comprehensive characterization and comparison of gene expression patterns in normal and diseased kidneys will provide novel targets for therapeutic intervention by identifying candidate pathways, which regulate nephropathy pathogenesis.

Sniper: Design of the high throughput fluorescent homogeneous assay for SNP scoring. *L. Kent¹, M. Kenrick¹, L. Bailey¹, S. Jones¹, I. Horsey¹, J. Briggs¹, T. Knott¹, J. Pickering¹, S. Randall², G. Nardone².* 1) Amersham Pharmacia Biotech, Amersham, England; 2) Intergen Discovery Products, Gaithersburg MD, USA.

A SNP scoring system has been developed based on allele specific amplification of polymorphic loci directly from genomic DNA. The Sniper™ system uses a technology that combines an allele specific probe ligation event, and Rolling Circle Amplification (RCA) - an isothermal mode of replication used to amplify target DNA. The homogeneous assay has been optimized for allelic discrimination using a single pair of Amplifluor™ primers labeled with different fluorophores for detection. Amplifluor primers are designed to generate a fluorescence signal only after the ligation discrimination event and the first round of rolling circle amplification. This allows for detection in a closed system, homogeneous microtitre plate format. The primers have been selected to enable their use as generic reporters to deliver simplicity and affordable use. Optimisation of synthesis and purification regimens for a variety fluorophore and quencher combinations has been performed. SNP scoring has been performed on both PCR products and nanogramme quantities of genomic DNA.

Identification of differentially expressed genes in hepatocellular carcinomas and metastatic liver tumors by oligonucleotide expression profiling. *D.T. Horne, A.J. Williams, D.J. Wilson, T. Eskandari, L.M. Vogt, J. Wetzel, J.F. Boland, J.G. Vockley.* Genomics R&D, Gene Logic, Inc., Gaithersburg, MD.

The multistep process of tumorigenesis involves a vast number of different genes. The changing expression levels of these genes cause the characteristic changes associated with the development of cancers. Using oligonucleotide microarrays, we have analyzed the gene expression profiles of three distinct groups of liver samples: normal liver samples, metastatic liver tumors, and hepatocellular carcinoma tumors. The patterns of gene expression for these three sample sets were compared in several ways to determine the genes and ESTs that are over- and underexpressed in hepatocellular carcinoma relative to normal liver expression, and those that are over- and underexpressed in metastatic liver tumors compared to normals. We have also identified the genes and ESTs that are expressed in the combined set of hepatocellular carcinomas and metastatic liver tumors, but not in the normal liver samples. These data confirm the involvement of some genes whose altered expression has previously been reported in cancers of various tissue types, not just in liver cancer. By determining the genes and ESTs that are differentially expressed in these tumors, we have identified potential targets for continued research for drug development, therapeutics, and other gene-based therapies for the treatment of liver cancer.

Development of a fifth-dye labeled short size standard for SNP interrogation. *S.S. Kuo, C. Chang, B. Rosenblum, R. Brown, S. Wong, M. Johnson, A. Tomaney, P. Dong.* Genetic Analysis, PE Applied Biosystems, Foster City, CA.

We describe the development of a short size standard for analyzing small fragments on fluorescent detection systems. This standard contains 9 fragments ranging from 15 to 120 nucleotides, and is labeled with a fifth dye. During rigorous testing with various media on gel or capillary electrophoresis platforms, we are able to achieve excellent precision and curve-fitting cross-platform. The fifth dye is spectrally well resolved from other dyes.

This standard is designed in particular to enable automated data analysis in methods for single nucleotide polymorphism (SNP) detection such as single-base extension (SNE) assays. The presence of the size standard will minimize the need for manual data analysis especially in multiplexed electrophoresis assays. In our poster, we will demonstrate its utility in SNE assays. With the combination of different fragment lengths and four-color chemistry, the potential for multiplexing SNP loci exists for large-scale genotyping with minimal optimization.

Pyrophosphorolysis activated polymerization (PAP): A highly specific method for analysis of rare somatic mutations. *Q. Liu, S.S. Sommer.* Molecular Genetics, City of Hope Natl Medical Ctr, Duarte, CA.

To detect minimal residual disease or to measure mutation load, a robust method for identifying one mutant allele in the range of 10^6 to 10^9 wild type alleles would be advantageous. Pyrophosphorolysis activated polymerization (PAP) has the potential to provide a highly specific and robust method of allele-specific amplification. In PAP, pyrophosphorolysis and polymerization by DNA polymerase are coupled serially by utilizing a pyrophosphorolysis activatable oligonucleotide (P*). P* is an allele-specific oligonucleotide with a dideoxy nucleotide at the 3' terminus that can be activated by pyrophosphorolysis to remove the 3' terminal dideoxynucleotide in the presence of pyrophosphate (PPi) and the precise complementary strand of the allelic template. The activated P* then can be extended by DNA polymerization. Specificity results from serial coupling of pyrophosphorolysis and polymerization since significant nonspecific amplification requires the combination of mismatch pyrophosphorolysis and misincorporation by the DNA polymerase, an extremely rare event which is estimated to be 3.3×10^{-11} . Systematic analysis of DNA polymerases, PPi concentrations, allele-specific templates, pH, and dNTP concentrations revealed conditions of sufficient efficiency to allow PAP to amplify specific alleles directly from human genomic DNA.

Scratching beneath the surface: Cluster-specific factor analysis of gene expression levels from cDNA microarrays reveals unique patterns. *L.E. Peterson*. Dept. of Medicine, Baylor College of Medicine, Houston, TX.

Recent advances in microarray technology have made it possible to simultaneously monitor expression levels of thousands of genes involved in cellular development, differentiation, proliferation, and response to environmental stimuli. With large data sets, cluster analysis typically identifies clusters containing hundreds of genes that can be further analyzed for patterns of expression. I describe results of exploratory factor analysis of cluster-specific gene expression data after UPGMA cluster analysis has been performed. Resemblance coefficients used in clustering were based on pairwise cosines of standardized input data. The principal component solution to the factor model of \mathbf{R} was solved for each cluster of genes identified. Factors whose eigenvalues exceeded unity were rotated using an orthogonal varimax rotation to achieve parsimony in the loadings. Results indicate that unique patterns of gene expression can be identified within clusters containing hundreds of genes. Algorithm output for simulated data will be presented in HTML format and includes JPEG images of clusters and expression levels, factor loading patterns, and plots of the mean and standard deviation of log(red/green) ratios for each unique set of genes identified. (Supported by NCI grant CA78199-02).

eGenome: Integration of complex genomic data into whole chromosome views. *E.P. Sulman¹, T.C. Matise², Z. Lu¹, C.J. Porter³, P.S. White^{1,4}.* 1) Division of Oncology, Children's Hospital of Philadelphia, PA; 2) Dept. of Genetics, Rutgers University, Piscataway, NJ; 3) Hospital for Sick Children, Toronto, ON, Canada; 4) Dept. of Pediatrics, University of Pennsylvania, Philadelphia, PA.

The integration of complex genomic data sets into a unified data structure is a crucial step towards identifying complex and poorly localized genetic disease genes. We have developed a method, eGenome, that combines radiation hybrid (RH), genetic linkage (GL), cytogenetic, and large-insert clone-based mapping data with transcript clusters and genomic sequence into a unified data structure accessible via straightforward Internet queries. Utilizing a relational database, eGenome incorporates data sets from diverse sources, including RHdb, CEPH Genotype database, dbSNP, UniGene, LocusLink, cytogenetic data from the Genome Database, and large-insert clone data. These data are assembled relative to a high-resolution, high-confidence physical framework and normalized to remove redundancies. When whole-chromosome sequence is available, as in the case of chromosomes 21 and 22, electronic PCR (ePCR) is used to construct the framework, providing exact positional information. Otherwise, RH marker frameworks are constructed to which a GL framework is aligned. All remaining RH and GL markers are placed in high-confidence intervals relative to the framework. Genomic elements sharing a common database identifier, such as a GenBank accession number, UniGene cluster ID, or D-Number, are grouped and assessed for mapping discrepancies and appropriate nomenclature assignments. Thus far, we have applied this process to completely sequenced (chromosome 22) and unfinished (chromosome 1) human chromosomes. Completion of the entire human eGenome project estimates integration of >150,000 genomic elements. Keyword and positional queries of eGenome are possible using marker names, cytogenetic band positions, or, for sequence-based maps, sequence position. Also, sequence may be entered for ePCR analysis and map placement. In addition to positional information, eGenome provides element-specific links to numerous online databases, including GenBank, dbEST, OMIM, and BLAST queries. eGenome is accessible at <http://genome.chop.edu>.

Performance characterization of cDNA microarrays produced by thermal ink-jet (TIJ) deposition. *L. Stanton¹, L. Bruhn¹, D. Weist¹, S. Lightfoot¹, H. Villaneuva¹, S. Collins¹, C. Sum¹, D. Ilsley-Tyree¹, P. Webb¹, M. Westall¹, C. Templin¹, S. Gonzalas¹, L. DaQuino¹, W. Fisher¹, K. Schleifer¹, R. Tella¹, J. Wohlgemuth², T. Quertermous², M. Caren¹, D. Amorese¹.* 1) Bioscience Products, Agilent Technologies, Palo Alto, CA; 2) Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, CA.

Microarray technology has rapidly developed as a versatile method to explore gene expression on a genome-wide scale. We have developed a process to print high-density arrays of cDNAs onto modified glass surfaces by Thermal Ink-Jet (TIJ) deposition. Our process has combined novel developments in glass surface chemistry, nucleic acid deposition, and image acquisition/analysis to produce high-quality cDNA arrays. These cDNA arrays are suitable for profiling gene expression of thousands of genes simultaneously. A solid understanding of system performance is essential for meaningful interpretation of expression data derived from complex biological situations. To characterize the performance of our system we have executed a series of controlled experiments that were designed to carefully measure the reproducibility, sensitivity, and reliability of expression data. We have determined the variance of expression signals produced within and between arrays by comparing values generated from replicate samples in replicate experiments. The lowest limits of detection for transcripts in this system and accuracy of differential expression values were determined by analysis of titrated reference RNA samples. In a functional test of our complete system, expression data were determined for several thousand human genes in a cell-based model of angiogenesis. These results show that cDNA microarrays produced by our TIJ deposition process are capable of generating highly reliable gene expression data.

Diagnosis of HNPP deletion and CMT1A duplication using real-time PCR. *K. Wilke, B. Duman, J. Horst.* Inst fuer Humangenetik, Westfaelische Wilhelms Univ, Muenster, Germany.

We report the development of a method for quantitation of the genomic copy number of genes. The method which we term GTEQ (genomic template number corrected quantitation by real-time PCR) involves separate PCR amplifications of a test locus with unknown copy number and a reference locus with known copy number. Progress of the PCR reactions is monitored using fluorogenic probes and a "real-time" fluorescence detection system. For each reaction, the number of cycles is measured at which a defined threshold fluorescence emission is reached. Using standard curves, the copy number of the test DNA relative to a common standard DNA is determined for each locus. From the ratio of the relative copy numbers, the genomic copy number of the test locus is determined. In order to demonstrate the accuracy and reliability of the method for genetic testing, we analyzed 43 patients with hereditary neuropathy with liability to pressure palsies (HNPP), containing a heterozygous deletion of a 1.5 Mb region on chromosome 17p11.2-p12, 8 patients with Charcot-Marie-Tooth disease, containing a heterozygous duplication of the same genomic region, and 50 normal control individuals. As a test locus we analyzed the PMP22 gene located within the 1.5 Mb region. The genomic copy number of the test locus was precisely measured, and the presence or absence of the genomic deletion or duplication was unambiguously diagnosed in all individuals.

Single copy hybridization probes derived by genomic sequence analysis. *P.K. Rogan^{1,2}, P. Cazcarro¹, J.H.M. Knoll^{1,2}.* 1) Sect Med Gen & Mol Med, Children's Mercy Hosp, Univ Missouri-Kansas City Sch Med 64108; 2) Cytogenomix, Overland Park KS 66213.

DNA hybridization probes complementary only to single copy sequence intervals were developed from previously determined human genomic sequences. The probes were used in hybridization analyses without adding repetitive sequence-blocking nucleic acids. The locations of single copy sequence intervals in known target sequences were determined by comparing these sequences with those contained in a comprehensive database of repetitive sequence families. DNA fragments suitable as hybridization probes were generated by long PCR amplification of the deduced single copy sequences. Purified single copy fragments were then labeled and hybridized to genomic sequences using either FISH (scFISH) or Southern analysis. Probes 2-6 kb long were developed from sequences at chromosomes 1p36, 15q12, and 22q11, respectively, from GenBank Accessions AL031282, AC006596, and NT_001039. A single, 2.3 kb labeled fragment was adequately and reliably visualized by FISH. Mixed combinations of DNA fragments from the same contig had discrete but more intense hybridization patterns on metaphase chromosomes compared to the same fragments applied separately. This finding validates scFISH for design and production of single copy probes.

To explore the feasibility of using these probes in high-resolution FISH studies, we determined the distribution of single copy sequences on chromosome 22. There are ~1507 single copy genomic intervals >2.3 kb in length, and significant clustering is evident (39% between 500-1000 bp apart). The minimum separation of single copy intervals is 35 bp and the maximum is 284 kb, with an average spacing of 22±31 kb. Except for 13 intervals on chromosome 22 separated by >150 kb, custom probes for high-resolution FISH analyses can be developed covering the entire euchromatic region. If single copy sequences are similarly distributed on other chromosomes, scFISH will enable more precise delineation of chromosome rearrangement breakpoints, distinction of multigene family members, and identification/sizing of marker chromosomes.

cDNA microarray analyses reveal differential expression of DNA repair-related genes among normal tissues.

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Wyrobek¹. 1) Biology & Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA; 2) Genetics and Developmental Biology Program, West Virginia University, Morgantown, WV.

The ability to efficiently repair DNA damage is essential for maintaining genomic integrity and health. The purpose of this research was to determine whether genes involved in DNA repair processes are differentially expressed among healthy adult tissues (testis, brain, liver, spleen and heart). We developed a custom cDNA microarray comprised of hundreds of genes representing various DNA repair pathways and functions: nucleotide excision repair, base excision repair, mismatch repair, homologous and non-homologous recombination, damage recognition, apoptosis, cell cycle and stress response. Total RNA was extracted from the tissues of B6C3F1 mice, reverse transcribed and labeled with fluorescent dUTPs, and hybridized onto the microarrays using pooled testes RNA as the reference tissue. Image analysis was conducted using software developed at LLNL. Our results indicate that genes in several DNA repair pathways (nucleotide excision repair, base excision repair, non-homologous recombination, etc.) are differentially expressed, with testis generally exhibiting the higher levels of expression. Genes showing elevated expression in the testis (2 to 29 fold) include *ercc1*, *pcna*, *DNAj*, *ho-2*, and *cdk-2*. A smaller set of genes exhibited elevated expression (2 to 7 fold) in the heart, spleen, liver and/or brain compared to testis including *tdg-b*, *paga*, *gpx1* and *cas1*. Most housekeeping genes had a 1:1 expression ratio among tissues. The finding that tissues differ in their baseline expression of DNA repair-related genes raises important questions regarding tissue-specific responses to genotoxic agents and differential genetic susceptibility to the onset and progression of cancer. [This work was conducted under the auspices of the U.S. DOE by LLNL under contract W-7405-ENG-48 with support from the NIH grant ES09117-02, DOE grant KP110202, the University of California at San Francisco and West Virginia University.].

SNP scoring by one base sequencing. *L. Spangberg¹, K. Erickson², J. Jonsson¹, P. Gad¹, J. Henriksson¹*. 1) Gemini Genomics AB, Uppsala, Sweden; 2) Amersham Pharmacia Biotech AB, Uppsala, Sweden.

There is an increasing demand of studying SNPs of the human genome for a range of different purposes. Many available SNP scoring techniques are relying on expensive dedicated equipment. The purpose of developing the one base sequencing (OBS) method was to use standard PCR and sequencing instruments. The OBS method uses a unique nucleotide mixture of three dNTPs and one ddNTP. An OBS primer is positioned adjacent or close to a SNP position. The extension from the OBS primer annealed to a single stranded PCR product continues until a ddNTP is incorporated. For example, for a G/C SNP and terminator ddGTP the extension will stop at the SNP if a G is present but will continue to the next G in the sequence if a C is present. A heterozygote sample will produce two extension products of different defined lengths. Cy5-labelled OBS primers, AutoLoad combs and different dNTP/ddNTP mixtures was used to analyse several different SNP with high performance on an ALFexpress instrument. A high multiplexing level of the OBS method is possible when OBS primers of different lengths are used for different SNPs. With five OBS primers of different length and six runs (reloading) the SNP scoring capacity reaches 1200 assays per instrument and day. In a triplex analysis of 1200 samples a success rate of over 97% was achieved data verified with full sequencing and mini sequencing. In order to reduce the bottlenecks, like data input and data analysis, a special software facilitating transferring data was written. This is important for minimising person-related errors. Ongoing development of the OBS method includes the use of alternative sequencing chemistry, software support of running and analysis and further automation of the sequencing procedures. In conclusion the OBS technique has several advantages, firstly it is accurate, both alleles of a SNP is studied in one reaction; secondly it is robust, with a very high success rates. Thirdly, the OBS method is less expensive, since it can be run on standard electrophoresis equipment and multiplexed. Finally, it has the potential for being useful for higher throughput platforms after automation and software support.

The Derivation of the Probability Distribution Function for Gametic Disequilibrium and a New Measure of Nonrandom Allelic Association. *B.A. Skierczynski¹, D. Cohen², C. Nguyen¹, N.J. Schork^{1,3}*. 1) Statistical Genomics, Genset Corp, La Jolla, CA; 2) Genset Corp, Paris, France; 3) Department of Epidemiology and Biostatistic, Case Western Reserve University, Cleveland, Ohio.

The detection of the nonrandom association of alleles (or "gametic disequilibrium" GD) at different loci is a powerful tool for both applied population genetic initiatives and the location of human disease predisposing genes. The magnitude of GD can be estimated from haplotype frequencies for a given population by means of a variety of disequilibrium coefficients. Although there are several different measurements of GD that have been proposed, none of these measures are completely independent of allele frequencies of GD which independent of allele frequencies. We previously derived this distribution using stochastic differential calculus but now extended our results to estimation and analysis issues. The proposed measure can differentiate the strength of GD between two population. Our derivations open a new way of characterizing GD and as such have great importance in both practical and theoretical contexts.

MiniSAGE: Gene Expression Profiling using SAGE from 1 mg Total RNA. *S.Q Ye, L.Q Zhang, F. Zheng, D. Virgil, P.O Kwiterovich.* Pediatrics, Johns Hopkins University, Baltimore, MD.

Complex diseases such as coronary heart disease, diabetes, cancer, whose etiology may be due to multiple genetic factors and their interplay with environmental risk factors, call for the new powerful techniques to identify the gene expression pattern changes in number of genes. Two highthroughput methods for monitoring of gene expression have been published: the DNA microarray and Serial Analysis of Gene Expression (SAGE). SAGE has an advantage of identifying the novel genes and providing absolute transcript numbers in a digital format over the former. A drawback of SAGE is the requirement of a large amount of input RNA (2.5-5.0 mg mRNA, which is equivalent to 250 to 500 mg total RNA). Several labs attempted gene profiling from a small amount of RNA using SAGE, but they were all involved either PCR amplification of starting cDNA materials or PCR reamplification of SAGE ditags generated by a first round of PCR amplification. These additional PCR amplifications potentially introduced bias and compromised the quantitative aspects of the SAGE method. We developed a miniSAGE technique and successfully carried out gene profiling of human fibroblasts from 1mg total RNA without extra PCR amplification. Three key modifications contributed to our establishment of miniSAGE: 1. application of Phase Lock Gel (Eppendorf) to purify DNA after phenol extraction in 7 steps in SAGE procedure to significantly increase the recovery of DNA material; 2. add of 25 fold less amount of linkers (10 ng/per reaction) ligating to cDNA to reduce its interference with SAGE ditag amplification and increase the SAGE ditag yield; 3. use of the mRNA Capture Kit (Boehringer Mannheim) to carry out first five steps of mRNA isolation, cDNA synthesis, enzyme cleavage of cDNA, ligating linkers to bound cDNA and release of cDNA tags in one tube to significantly reduce the loss of material between successive steps. Our preliminary analyses of 1100 tags each from two human fibroblast libraries made using miniSAGE demonstrated typical fibroblast gene expression patterns, which are similar to that made by standard large scale SAGE in our lab. This miniSAGE will broaden the applications of SAGE.

Direct full-length cloning of mammalian mRNAs using a novel library-free method mediated with ligation anchored cDNA. Z. Xu, C. Xia, K. Eng, L. You, X. Yuan, D. Jablons. Cancer Center, Department of Surgery, School of Medicine, UCSF, San Francisco, CA.

Generation of full-length sequenced cDNAs is of great importance in gene discovery and functional analysis. Current technologies for obtaining full-length cDNA clones are tedious and time consuming. We have developed a novel library-free method for rapid full-length cDNA cloning of mammalian genes. The method involves ligation of an anchor sequence to the 3' end of the first strand cDNA after reverse transcriptase reaction. A gene-specific first strand cDNA was isolated with a biotinylated primer and streptavidin coated magnetic beads. Gene-specific full-length cDNAs were then amplified using high fidelity DNA polymerase and directly cloned into plasmid vectors. We have successfully used this method for cloning several representative full-length cDNAs from RNA samples isolated from human cell cultures. The full-length cDNAs isolated using this method include the tumor suppressor p53 (2.5 kb), transferrin receptor (5 kb), cystic fibrosis transmembrane conductance regulator (CFTR) (6.2 kb), and an anonymous lung cancer specific cDNA (1.8 kb). Sequence analysis showed that the complete full-length clone for p53 is 2,562 bp in size with a 54-bp longer 5' end sequence and 820-bp more sequence at 3' end than the published p53 cDNA clone (1.7 kb). The transferrin receptor cDNA clone consists of 5,032 bp, and has a 20-bp longer sequence at 5' end and 222-bp more sequence at 3' end. The 5' end sequencing of the CFTR full-length cDNA clone showed a 3-bp longer sequence than the published data. The complete insert of an anonymous lung cancer specific cDNA clone is under sequencing. Our results demonstrate that this library-free technology is more efficient than the conventional methods for cloning full-length cDNAs that carry intact 5' and 3' untranslated regions in addition to protein open reading frame (ORF). (Supported partially by NIH grant R21CA85172).

The identification, genomic structure and screening of a H⁺ transporting ATPase gene within the X-linked cone-rod dystrophy critical region (Xp11.4). *F.Y. Demirci^{1,2}, N. White³, X. Chun-Fang³, M.B. Gorin^{1,2}*. 1) Dept. of Ophthalmology, Eye and Ear Institute, University of Pittsburgh, Pittsburgh, PA; 2) Dept. of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 3) Physical Mapping Unit, UK Molecular Genetics, Glaxo Wellcome, England.

Our group refined the X-linked cone-rod dystrophy (COD-1) locus to a limited region of Xp11.4 between the RP2 and RP3 loci, ~ 0.5 cM. A complete YAC/BAC/PAC physical contig of the COD-1 region was used for high throughput sequencing and BLAST searches to identify homologies with ESTs within Genbank. Those ESTs were analyzed for overlapping or related cDNA sequences and particular attention was made to ESTs that were initially derived from a retina cDNA library. One of the candidate genes within our region encodes a H⁺ transporting ATPase (APT6M8-9) matching 12 of the ESTs (one from a retina cDNA library) and also listed in Genbank under HSM800272. The cDNA transcript was compared with the high throughput genomic sequencing results to specify intron-exon boundaries using MacVectorTM6.5, AssemblyLIGNTM and Sequencher 3.1.1 software. We have identified 8 exons (with flanking intronic sequences) using the published partial cDNA sequence and genomic sequence data. We screened for mutations in COD-1 affected males using PCR amplification and direct sequencing of these exons (including exon-intron junctions) from genomic DNA. No causative mutations have been identified. We are completing the missing 5' end full length sequence of this gene by isolating clones from a retina cDNA library and using the BAC/PAC clones from within our contig. So far, we have identified at least one additional exon, which is not present in the published sequence and more upstream exons may be present. Support: The Eye & Ear Foundation of Pittsburgh (MBG), Research to Prevent Blindness, Inc. NY., NY.

Physical and transcription map of the SMS critical interval. *R.E. Lucas¹, C.N. Vlangos¹, P. Das³, P.I. Patel³, S.H. Elsea^{1,2}.* 1) Genetics Graduate Program, Michigan State University, East Lansing, MI; 2) Depts. of Zoology and Pediatrics/Human Development, Michigan State University, East Lansing, MI; 3) Dept. of Neurology, Baylor College of Medicine, Houston, TX.

Smith-Magenis syndrome (SMS) is a multiple congenital anomalies/mental retardation syndrome associated with an interstitial deletion involving chromosome 17p11.2. SMS is believed to be a contiguous gene syndrome in which the haploinsufficiency of multiple, functionally-unrelated genes in close physical proximity leads to the characteristic behavioral and physical phenotype. We recently defined the ~1.5-2 Mb SMS critical deletion interval between marker D17S29 (centromeric) and cosmid cCI17-638 (telomeric). We now report a physical and transcription map of the SMS critical region, consisting of overlapping bacterial artificial chromosome (BAC), P1 artificial chromosome (PAC), and cosmid clones. The deletion region is very gene-rich, with 13 genes, 12 ESTs, and 10 genomic markers mapped to the BAC/PAC/cosmid contig by Southern blotting, restriction pattern analysis, direct cosmid sequencing, and examination of high-throughput genome sequence information. Marker position in the contig was related to natural, overlapping deletions along 17p11.2 by PCR of DNA from rodent:human somatic cell hybrids that retain a deleted chromosome 17 from SMS and non-SMS patients. Several of the genes that were mapped have been well-characterized, but many of the ESTs that lie within the critical interval have not yet been studied. In order to identify a promising candidate gene for SMS, we sequenced the inserts from the ESTs and determined their expression patterns and transcript size by northern blot hybridization. Extensive BLAST searches and sequence analyses reveal that most of these ESTs are novel, and further studies are underway to determine the role these genes may play in the SMS phenotype.

Organization of satellite I sequences on human chromosome 21. *M. Roy¹, J. So¹, M. Cummings², J. Doering¹.* 1) Dept. of Biology, Loyola University Chicago; 2) Dept. of Biological Sciences, University of Illinois at Chicago.

The human genome sequence will not include centromeres and other heterochromatic regions, although these sequences comprise 10-15% of the genome. We are constructing a detailed physical map of the centromere and p arm of chromosome 21 as a model for the organization of these regions. The satellite I (sat I) repetitive sequence family is found in heterochromatic regions on all acrocentrics and chromosomes 3 and 4. We recently identified a new sat I subfamily, L2, that shares 80% sequence identity with pTRI-6, a previously-characterized sat I subfamily. While pTRI-6 is found predominantly on chromosome 13 with a small amount on chromosome 21, L2 is located on chromosomes 3 and 4 and all of the acrocentrics. Using a chromosome 21 hybrid cell mapping panel, we regionally mapped both sat I subfamilies on this chromosome. L2 is located solely on the p arm distal to the rDNA cluster, while pTRI-6-like sequences are present both in the p arm proximal to the rDNA and in the centromere. The centromeric sat I cluster and adjacent regions of chromosome 21 were physically mapped. DNA from 153E7BX, a hybrid cell line which contains the long arm and centromere of chromosome 21, was restricted to create large fragments, subjected to pulsed field electrophoresis and probed with pTRI-6. This sat I cluster is 0.22 Mb long and located 0.28 Mb from the q arm end of the major alphoid cluster, D21Z1. Internally, the sat I cluster consists of tandemly repeated pTRI-6-like sequences periodically interrupted by other sequences that are either unique elements or members of another sat I subfamily. This centromeric sat I region is not found on other chromosomes, and its size and internal organization are polymorphic in the population. Given the chromosome 21 specificity, polymorphism and unique organization of the centromeric sat I cluster, sequences within this region are candidates for a chromosome 21 centromeric marker that could be used to definitively score meiotic stage and parent of origin for non-disjunction. Currently-used markers on the pericentromeric q arm sometimes give erroneous results because they undergo recombination relative to the centromere.

Transcript mapping of the BPES critical region at 3q23: identification of novel candidate genes. *E. De Baere, L. De Vuyst, A. De Paepe, L. Messiaen.* Department of Medical Genetics, Ghent University Hospital, Ghent, Belgium.

The blepharophimosis syndrome (BPES) is a rare genetic disorder characterised by blepharophimosis, ptosis, epicanthus inversus and telecanthus. In BPES type I, BPES is associated with female infertility, while in BPES type II the craniofacial defect occurs isolated. The BPES syndrome has been mapped to 3q23, but a linkage analysis which showed linkage to 7p provided evidence for locus heterogeneity. By mapping YACs, PACs and cosmids surrounding the 3q23 translocation breakpoint of a t(3;4)(q23;p15.2) BPES patient we narrowed down the critical interval to 45-kb.

Recently, we identified a novel candidate gene (*BPESCI*, BPES candidate 1) which was shown to be disrupted by the 3q23 translocation. Since mutation analysis in other BPES patients revealed no causative mutations, the functional role of *BPESCI* in the pathogenesis of BPES is still unclear. *BPESCI* may reside in a large intron of another gene or BPES may be caused by a position effect in this patient.

Thus, transcript mapping of a broader interval around the breakpoint (D3S1615-D3S1316) was performed by the integration of GeneMap'99 ESTs in an existing framework of YACs and PACs. BLAST (htgs) analysis of mapped ESTs revealed similarity to partially sequenced BACs from this region. These partial contigs were analysed using a range of programs from the NIX database (<http://www.hgmp.mrc.ac.uk/NIX>) leading to the identification of several known and novel candidate genes/ ESTs. Genes/ ESTs expressed in tissues which might be important in BPES (ovary, fetus) are further analysed preferentially as candidates for BPES.

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Characterization of 41 genes identified through the analysis of 1 Mb of sequence surrounding the Van der Woude syndrome (VWS) critical region at 1q32. *B.C. Bjork¹, B.C. Schutte¹, Y. Watanabe¹, E.L. Hockenhull², M.I. Malik¹, M.J. Dixon², B. Yuan³, J.C. Murray¹.* 1) Dept. of Pediatrics, Univ. of Iowa, Iowa City, IA; 2) Univ. of Manchester, Manchester, UK; 3) Ohio State Univ., Columbus, OH.

VWS is an autosomal dominant form of clefting with bilateral lower lip pits. The VWS gene was localized to a 1.6 cM region of 1q32 between D1S491 and D1S205. The sequence for 1.1 Mb surrounding the 350 kb VWS critical region is available in two sequences of 721 kb and 269 kb, separated only by a 100 kb gap. Gene identification was performed using a suite of similarity searches and gene prediction programs included in a modified version of Genotator. Five gene prediction programs were used to identify putative exons. RepeatMasker2 was used to identify and "mask" repetitive sequences before performing BLAST homology searches against various publicly available nucleotide, est and protein databases. These analyses revealed 4 'known' genes, 14 'related' genes, 20 'predicted' genes, 3 pseudogenes and an additional 49 exons predicted by at least 2 gene prediction programs. 75 of 94 exons (80%) from 11 known and related genes were predicted by 2 or more gene prediction programs. In addition, from these analyses we estimated values for the following genomic characteristics: minimum gene density (37 per Mb), gene size (min., 900 bp; max., >157 kb; avg., 36 kb), exons per gene (min., 2; max., 23; avg., 10), exon size (min., 30 bp; max., 6177 bp; avg., 275 bp), intron size (min., 66 bp; max., 73.2 kb; avg., 3.89 kb) and intergenic space (min., 5 kb; max., 81 kb; avg., 36 kb) all of which compare reasonably to other estimates derived for the human genome. All 'exon' sequences are screened for the presence of etiologic mutations in a panel of 90 affected individuals by SSCV analysis and direct sequencing. To date, we have analyzed 106 exons and have identified 25 sequence variants, but none of these are etiologic mutations. We continue to prioritize all related and predicted genes for mutation screening by obtaining cDNA sequence and expression data.

Gene expression changes in endometrial cancers. *M. Mahadevappa*¹, *F. Du*², *M. Durst*¹, *A.M. Bowcock*², *J.A. Warrington*¹. 1) Health Management, Applied Research, Affymetrix, Inc., 3380 Central Expressway, Santa Clara, CA 95051; 2) Dept. of Genetics, Pediatrics & Medicine, Div. of Human Genetics, Washington University School of Medicine, Campus Box 8232, 4566 Scott Avenue, St. Louis, MO 63110.

Endometrial cancer is the most common cancer of the female reproductive system and is the fourth most frequently diagnosed cancer among women in the US. Adenocarcinomas, cancer of glandular cells, comprise more than 75% of endometrial cancers. Clear cell carcinomas, cancer of epithelial cells, comprise less than 5% of endometrial cancers. Matched normal tissue and adenocarcinoma or clear cell carcinomas were collected from 10 patients. Total RNA was used as starting material for the preparation of labeled targets from all of the samples. The labeled targets were hybridized to high-density DNA microarrays containing probes representing ~6800 full-length human genes. Differential gene expression patterns in both of the subtypes of endometrial cancer were identified using a number of data analysis tools including GeneChip® software, GeneCluster, GeneSpring and GeneExplore. A number of genes exhibited profound expression differences. For example, macrophage migration inhibitory factor (MIF) and cyclin A1 transcripts were detected in adenocarcinomas and undetected in normal tissues. MRG1, melanocyte-specific related gene, and HOX1, a homeobox gene, were detected in normal tissues and undetected in adenocarcinomas. There were also significantly lower transcript levels of alpha2 type VI collagen and adducin, and significantly higher transcript levels of cyclin B and protein kinase C zeta in adenocarcinomas. In the case of clear cell carcinomas, transcripts detected in normal tissues such as calponin and caldesmon were not detectable. However, these tumor types exhibited expression of type II keratin K17, epithelial specific transcription factor (ESE-1b) and high mobility group (HMG1) genes. Expression of these genes was not detected in normal tissue. Increased expression of LAMB3, laminin S B3, and osteopontin and decreased expression of decorin and adducin genes were noted in clear cell carcinomas when compared to expression levels in normal tissues.

North American Indian Childhood Cirrhosis (NAIC or CIRH1A): physical mapping and *in silico* identification of candidate genes. A.M. Richter¹, P. Chagnon¹, E. Drouin², A. Rasquin-Weber², G.A. Mitchell¹. 1) Medical Genetics; 2) Gastroenterology, Hôpital Sainte-Justine, Montréal, Canada.

NAIC/CIRH1A (MIM 604901) is an early onset, familial cholestasis, frequent in the Ojibway-Cree population of the Abitibi region in north-western Quebec, Canada. The disease typically presents with transient neonatal jaundice in a child who is otherwise well, and progresses to biliary cirrhosis requiring hepatic transplantation in childhood or young adulthood. Clinical and physiological investigations have failed to discover the underlying cause of NAIC. Genealogies are consistent with autosomal recessive transmission and gene carrier frequency has been estimated at 9%. Recently, using DNA samples from 5 NAIC families, we mapped the NAIC locus by homozygosity to chromosome 16q22. High-density mapping identified a five-marker haplotype (*D16S3067*, *D16S752*, *D16S2624*, *D16S3025* and *D16S3106*) spanning 4.9 cM that is shared by all affected individuals (Bétard et al. Am J Hum Genet 67:222-228, 2000). As a prerequisite to cloning of the NAIC gene we are creating a large-scale physical and transcript map of the linked region by adopting an *in silico* strategy combined with traditional physical mapping. With the help of collaborators at DOE-JGI, we identified 82 BACs mapping to the NAIC linked region. For the majority, sequencing is incomplete, but large amounts of unassembled sequence are available in public databases. Other databases, such as LDB, <http://cedar.genetics.soton.ac.uk/>; UDB and GeneCard <http://bioinformatics.weizmann.ac.il>, GeneMap, <http://www.ncbi.nlm.nih.gov>, and the use of the Sequencer software to create virtual contigs, made it possible to identify the position of 55 genes with known function within the linked region. Following close scrutiny of their function and expression patterns we targeted 5 as candidate genes for NAIC. Sequencing of these genes in patients and controls is in progress. As the *in silico* strategy targets only genes with known function, in parallel we are proceeding with classical methods of physical mapping, using YACs and BAC/PACs to place STSs and ESTs in the region. Additional meiotic mapping and recruitment of other patients is also underway.

Multivariate association analysis of immune systems parameters and HLA polymorphism. *T.W. Chu¹, R.A. Norman¹, L. Essioux², B. Thiel³, J. Lanchbury⁴, M.A. Schork¹, B. Winkelmann⁵, N.J. Schork¹.* 1) Statistical Genomics, Genset Corp., La Jolla, CA; 2) Genset, SA, Paris, France; 3) Case Western Reserve University, Dept of Epi-Biostat, Cleveland, OH; 4) Guy's King's and St. Thomas' School of Medicine, Dept of Rheumatology, London, England; 5) Frankfurt University, Dept of Cardiology, Ludwigshafen, Germany.

The relationship between HLA polymorphism and disease is well documented but complex. Analysis methods designed to draw inferences about the contribution of HLA loci to disease susceptibility must therefore accommodate these complexities. We have developed a multivariate suite of methodologies to consider relationships between parameters characterizing complex physiological systems as dictated, in part, by the pleiotropic effects of genes. These methods have the ability to show precisely how a system of phenotypic endpoints, like those which describe the functioning of the immune system, are affected by genetic variation among individuals. We describe the derivation of our methods as well as their application to an extremely large data set consisting of a number of immune system phenotypes and HLA data collected on over 2000 individuals. Our proposed methods have the potential to contribute to human functional genomic initiatives of the type likely to be promoted in the post-genome sequencing era.

A High Resolution 7Mb Transcript Map of the Type 2 Diabetes Susceptibility Locus on Human Chromosome 20.

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Recent linkage studies and association analysis suggest the presence of at least one Type 2 diabetes gene on human chromosome 20q12-13.1 (e.g. Bowden et al., Diabetes 46, 882-886, 1997; Ghosh et al., P.N.A.S. U S A 96(5), 2198-2203, 1999; Price et al., A.J.H.G. 61(Suppl) A241, 1997). To facilitate efforts to clone the diabetes gene(s) in this interval, we have ordered 62 BAC clones from the Human CITB BAC Library (Research Genetics, Huntsville, AL.). A BAC Map was constructed using STS/marker-based screening methods from 130 markers (23 known genes, 70 ESTs, 16 STSs, 21 polymorphic markers). A contiguous assembly of markers and BAC clones was generated in semi-automated fashion with our GraphMap software program, that employs a greedy acyclic graph algorithm supplemented with local heuristics to determine optimal clone assembly. Two independent ordering checks were performed. Marker sequences were aligned with genomic sequence data released by the Human Genome Project (primarily the Human Chromosome 20 Sequencing Team at the Sanger Centre), and BAC end sequences were used to bridge sparsely sequenced regions to corroborate the putative marker/clone assembly. The marker content of each BAC clone was also correlated with retention patterns observed in a panel of 24 local overlapping yeast artificial chromosome (YAC) clones (Price et al., 1999 Genomics 62, 208-215.) creating a genomic scaffold spanning the region. The integrated map reveals three small gaps (less than 100kb) within the 7Mb BAC transcript map; regions in which minimal genomic sequence data is available and few STSs have been identified. The combination of the BAC transcript map, YAC scaffold and reference Human Genome Project Sequence provides a powerful integrated resource for future genomic analysis of this region. To date, we have evaluated 12 candidate genes and 3 novel ESTs for allelic variants associated with Type 2 diabetes, including HNF-4a, a protein kinase C binding protein (PRKCBP1) and a novel glucose transporter.

LDB2000: Sequence-based map integration. *A. Collins, W.J. Tapper, X. Ke.* Dept Human Genetics, Univ Southampton, Southampton, England.

With the majority of the human genome sequence now available it is possible for the first time to integrate different types of genome map where the order of almost all map objects is known without error. The location database (LDB), which has long been available at http://cedar.genetics.soton.ac.uk/public_html/, is being updated (as LDB2000) to include sequence-based genetic maps. As part of this effort we are locating in sequence genes, EST clusters and polymorphic markers (including SNPs) that have already been mapped by a range of methods (linkage, radiation hybrid, FISH etc.). Genetic linkage maps developed from CEPH and other linkage data are being re-built for known order and an algorithm for relating cytogenetic bands to sequence has been developed. For the first time recombination hotspots can be characterised with some confidence. Analysis of chromosome 22q has shown five broad regions of elevated recombination. In males the most striking area of elevated recombination is near the telomere where there are approximately 11 cM/Mb and in females there is a mid-arm region with 8 cM/Mb. Chiasma maps developed from spermatocytes show generally good agreement with the male linkage map although are somewhat shorter perhaps reflecting the effect of residual typing error in the (error filtered) male map. The resolution of the genetic map can be improved by locally re-distributing any clustered loci according to their sequence position. The use of genetic rather than physical map distances in multilocus methods to map disease genes by linkage disequilibrium has been shown to greatly increase precision where the region shows unusually high or low recombination. We anticipate that these sequence-based integrated maps will be most useful for this purpose. Although there is some suggestion of a relationship between R bands and increased recombination and radiation sensitivity analysis of further sequenced chromosomes should offer more insight into these relationships.

Making Sense of the Chromosome 3 Draft Sequence. *D.K. Garcia¹, K. Jastrow¹, X. He¹, T. Martinez¹, G. Barerra¹, S. Scherer², R. Gibbs², S.L. Naylor¹.* 1) Cellular & Structural Biology, U of Texas Health Science Ctr, San Antonio, TX; 2) Baylor College of Medicine Houston, TX.

The acceleration of the time table for producing a draft sequence of the human genome required a strategy for quickly isolating clones. We compiled entry clones (seeder clones) from several different sources. First, 640 markers at approximately 1.3cR intervals on the GB4 panel were chosen. Of these, clones were found for 480 markers using the RPCI11 segments 1-4. There are consistent regions that have no clones in this library - some of which were also noted on the DeJong database. Even at a distance of 1.3cR between markers, some clones were identified by more than one marker while other markers failed to identify any clones. These clones were supplemented with clones from other sources - 23 clones identified by FISH by Barb Trask and 68 clones FISH mapped by Barb Trask and identified by Pieter DeJong as corresponding to Genethon markers and 232 clones identified by matching sequences from flow sorted chromosome 3 library to RPCI11 and CalTech BAC end sequences. An additional 78 clones radiation-hybrid mapped to chromosome 3 by David Cox and fingerprinted by Washington University, have been added to the queue. All of the above clones which mapped back to chromosome 3 and positioned on the GB4 panel in gaps left by the initial screening are being sequenced. The seed clones created "backbone" of clones ~400kb apart has been created. For the q arm of chromosome 3 a high density screening had been completed and we were able to employ these data to construct contigs of the long arm that are consistent with the fingerprint contigs found in the Washington University fpc database. However, for the short arm of chromosome 3, clones were identified strictly by fingerprint. As data has accumulated, we have verified the maps of chromosome 3 using e-PCR and BLAST of mapped markers. The long arm of chromosome 3 is primarily being sequenced by Baylor while the short arm of chromosome 3 is being sequenced by Washington University, the Beijing Genome Center, and the Whitehead Institute. Our integrated map of the entire chromosome is posted on our website (apollo.uthscsa.edu).

Association between GT Microsatellite Repeats and Recombination in the Human Genome. *J.A Majewski, J. Ott.*
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Comparisons of genetic and physical maps of human chromosomes reveal the existence of distinct regions where meiotic recombination is either enhanced or suppressed, relative to its mean rate. Such recombination hot spots and cold spots have already been observed in experimental model organisms, and it is believed that primary and secondary DNA sequence, as well as chromatin structure and associated proteins, influence the frequency of recombination within a specific region. The recent availability of complete sequences of human chromosomes 21 and 22 allows us to correct genetic maps previously obtained from CEPH families, and to investigate the association between recombination frequency and specific DNA sequence patterns. We demonstrate significant correlation between the distribution of long tandem GT repeats, known to form Z-DNA and interact with several components of the recombination machinery, and recombination hot spots on both human chromosomes 21 and 22. In particular, the analysis of chromosome 21 data indicates that the observed association may be exclusively due to male-specific recombination. The existence of the relationship on both chromosomes suggests that the association between GT microsatellites and recombination may be universal throughout the human genome.

Chromosomal Assignment: A low resolution BAC map of the human genome. *J. Sainz, S. Gudjonsson, J. Hauksson, H. Magnusson, J. Arnadottir, K. Mylonas, N. Vidarsdottir, O. Traustason, H. Ingjaldsson, E. Juliusson, K. Stefansson, J. Gulcher.* Decode Genetics, Reykjavik, Iceland.

We are assigning bacterial artificial chromosomes (BACs) to 48 chromosomal regions with an average size of 69 Mb (from 31 Mb to 105 Mb) spanning the complete human genome. BACs are associated to pools of markers of known physical location. At present we have mapped, at this resolution, 2,805,000 Kb or 85.3% of genome. We have assigned 35,995 BACs to 41 genomic regions including chromosome 1 to chromosome 16 and chromosome X. On average, we detected 14.3 BACs per Mb equivalent to 2.4 X coverage of the analyzed genome. The BACs were detected using 16,514 genetic markers, with known physical locations on the Unified Database from Weizmann Institute, or a density of one marker per 168 kb of genome. The primers from the markers were used to screen a RPCI-11 BAC library by hybridization. To minimize the number of positive BACs due to repeated sequences or non-specific signals: 1) The primers were analyzed and discarded according to their content of *E. coli*, vector or repeated sequences; 2) We used a coincident-hybridization method based on two pools of primers, forward and reverse, and selected only the clones giving signals in both hybridizations. We assigned 1,490 BACs (4.1%) to more than one chromosome, very likely because they contain homologous regions in the genome or they contain repeated sequences with homology to the markers. The BACs assigned are being re-arrayed according to their region, gridded and screened by single marker hybridization. During the last five months, we have performed 10,432 single marker hybridization to construct fine resolution physical maps. The data generated by this chromosomal assignment has also proven to be very useful in combination with the fingerprinting BAC data (FPC) from the Sanger Center and Washington University, and the draft sequences from the Human Genome Project.

Microarray Analysis of Human Mitochondrial Disease Patients. *K.W. Kerstann¹, V.F. Procaccio¹, H.C. Yen², S.H. Hosseini¹, P.Z. Golik¹, D.C. Wallace¹.* 1) Center for Molecular Medicine, Emory University School of Medicine, Atlanta, GA; 2) School of Medical Technology, Chang Gung University, Kwei-Shan, Tao-Yuan 333, Taiwan.

We are using DNA microarrays to profile changes in gene expression in mitochondrial diseases. Through data mining, we have identified and collected 590 human cDNAs which are involved in energy metabolism, oxidative stress, and apoptosis. These are arrayed on glass slides and hybridized to differentially labeled cDNAs from patients and controls. This human MitoChip has been used to analyze changes in gene expression in two Leighs syndrome patients. One patient has a respiratory complex I defect in skeletal muscle and cultured cells, and was found to be a compound heterozygote for two missense mutations in the 23 kD NADH dehydrogenase (NDUFS8) gene of complex I. The second patient had a typical complex IV cytochrome c oxidase deficiency associated with SURF-1 gene mutations. This child was from a consanguineous marriage and was homozygous for a nonsense mutation in the SURF-1 gene. Expression profiling of muscle and cultured cell RNAs revealed that the mRNA levels of the 23 kD NDUFS8 gene were not significantly altered in the patient. However, many nuclear and mitochondrially encoded complex I genes were down regulated; including the mitochondrial DNA (mtDNA) transcripts ND4, NDL4, and ND6. The mRNA profile of the SURF-1 gene patient revealed a marked reduction in the SURF-1 transcript, consistent with nonsense mediated decay; as well as the down - regulation of multiple genes including SOD2, 70 kD heat shock protein, VDAC4, adenine nucleotide translocase 2, and glutathione peroxidase 3. These results suggest that DNA microarrays may provide an important diagnostic approach for screening patients for possible mitochondrial disease.

High throughput complete sequence screening of the dystrophin cDNA reveals a common duplication mutation, and over diagnosis of dystrophinopathy in Becker Dystrophy. *J.R Gorospe^{1,2}, S.A. Hamed¹, A. Sutherland-Smith³, J. Kendrick-Jones³, E.P. Hoffman¹.* 1) Research Center for Genetic Medicine, Childrens' National Medical Center, Washington, DC; 2) National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 3) Structural Studies Division, Medical Research Council Laboratory of Molecular Biology, Cambridge, UK.

We report automated capillary gel analysis of the complete 11 kb coding sequence of the dystrophin RNA from 38 patient muscle biopsies. 15 deletion-negative Duchenne Dystrophy patients were analyzed using this approach; mutations were identified in 80% of patients (12/15) and included novel premature stop codons, small deletions, and a recurring duplication of exon 2. The latter accounted for 25% of deletion negative DMD patients and is the most common dystrophin gene mutation which, surprisingly, has not been previously reported. 23 deletion negative Becker dystrophy patients were also analyzed. Of 18 patients who were thought to have unambiguous clinical and biochemical findings of Becker dystrophy, 40% showed causative dystrophin gene mutations (7/18). None of the 5 patients with more questionable clinical findings of Becker dystrophy showed mutations (0/5). Seventy percent (11/16) of patients with decreased quantities of normal molecular weight dystrophin showed no mutation by complete sequence analysis, suggesting that many may have some other disorder with secondary reduction of dystrophin. We identified 7 novel mutations in this study, including 2 missense in the amino-terminal actin binding domain (L172H, R82P), both of which fall outside known protein binding sites but both are shown to disrupt the tertiary structure of the protein through placement on the X-ray crystallographic map. Quantitative studies of dystrophin mRNA in 17 of the patient biopsies showed normal levels in the majority of mutation-negative patients tested. Our findings suggest that dystrophin protein studies must be interpreted with caution in deletion-negative male muscular dystrophypatients, as most patients showing decreased quantities of normal molecular weight dystrophin will show no detectable gene mutation.

Pyrosequencing: Performance and application to Factor V Leiden determination. *A. Alderborn¹, W. Strandberg¹, I. Muldin¹, C. Wadelius², A. Tallsjö¹.* 1) Pyrosequencing AB, Uppsala, Sweden; 2) Dept of Genetics and Pathology, University of Uppsala, Uppsala, Sweden.

PyrosequencingTM is a real-time sequencing method based on synthesis detection. Pyrophosphate (PPi), released during nucleotide incorporation by DNA polymerase, is detected as light produced by an enzyme cascade system. Pyrosequencing AB (Uppsala, Sweden) recently introduced the PSQ 96TM System, in which 96 different Single Nucleotide Polymorphism (SNP) samples are analyzed in parallel in less than 15 min. All reagents needed for performing a Pyrosequencing analysis in the PSQ 96 System are provided in an SNP Reagent Kit (enzymes, substrates and nucleotides), and dedicated SNP software delivers genotype and a quality assessment for each sample. Template preparation for analysis begins with a standard PCR reaction, followed by immobilization onto magnetic beads, denaturation by alkali and annealing of a sequencing primer. We have shown that the PSQ 96 System is highly flexible and robust with respect to positioning of the sequencing primer relative to the SNP position as well as PCR template quantity and quality requirements. When a specific template were sequenced with separate sequencing primers on different distances from the SNP, identical results in SNP genotyping were achieved. Different amounts of PCR product used for the template preparation also gave the same result in genotyping. Furthermore, PCR reaction products from different amplifications gave the same result when genotyped, demonstrating flexibility with respect to PCR amplification, storage of PCR products and success of the PCR reaction. The G1691A mutation in the coagulation factor V gene (Factor V Leiden) is the most common cause of resistance to activated protein C. Due to the increased risk for venous thrombosis caused by this mutation, the genotyping of this position has become clinically important. We have investigated this mutation by Pyrosequencing. All three genotypes were easily distinguished and correctly determined, proving that Pyrosequencing offers a reliable method for genotyping of the Factor V Leiden mutation.

Homogenous fluorescent assays for detection of single nucleotide polymorphisms by allele-specific amplification utilizing novel fluorescent primers. *M. Darfler, H. Jordan, A. Gallego, I. Nazarenko, I. Bouris, A. Rashtchian, J. Solus.* Molecular Biology Research and Development, Life Technologies Inc, Rockville, MD.

Methods that can easily and inexpensively genotype SNPs in samples will facilitate their detection and analysis. We have developed homogenous fluorescent assays based on the use of oligonucleotides labeled with a single fluorescent dye that produce a large increase in fluorescence intensity when incorporated into double stranded DNA. We show the use of this unique primer design to detect point mutations using several formats and SNP targets.

These primers were used in allele specific PCR amplification where homogenous phase detection of the allele specific products is demonstrated by real-time analysis of the PCR or by endpoint analysis in a fluorescence plate reader after the PCR amplification. Alternatively, multiplex PCR of up to 7 SNPs have been amplified in a single reaction and the fluorescent products resolved on an automated sequencer. This format, although not utilizing the potential for homogenous phase detection, allows higher throughput and reduces the time, labor, and cost of analyzing multiple targets.

The specificity of the PCR will have an effect on the ability to discriminate single base changes in the samples being analyzed. The effect of changes in the design of the allele specific primer for improved discrimination is shown. We also demonstrate the use of a novel DNA polymerase which increases the specificity of the allele specific PCR amplification. This DNA polymerase has been pre-complexed with monoclonal antibodies which inhibit the polymerase activity until the reaction is heated to high temperature. This confers added specificity to the start of the PCR, allows an automatic hot start of the reaction and permits setup at ambient temperature, facilitating the setup of large numbers of samples. The combination of improved primer design, a novel DNA polymerase and homogenous phase fluorescent detection provide a simple and reliable method for genotyping of SNP targets.

Genomic Variation Screening: a new method to identify, characterize and quantitate variations in isolated and pooled nucleic acid templates. *E.P. Dawson*¹, *J.A. Phillips III*², *L.C. Layman*³. 1) BioVentures, Inc, Murfreesboro, TN; 2) Vanderbilt University School of Medicine, Nashville, TN; 3) Medical College of Georgia, Augusta, GA.

As human and other genomic sequences become available, better methods to detect sequence variations will be needed to enable detection of sequence variations that may contribute to disease susceptibility or resistance or other traits. We have devised a new method, referred to as genetic variation screening or GVS that overcomes some of the limitations of DNA sequencing, especially detection of heterozygosity. GVS can simultaneously identify, characterize and quantitate sequence variations in isolated and pooled nucleic acid templates. GVS utilizes labeled primers, dNTPS and/or dideoxy terminators to obtain products that represent the entire nucleotide sequence of both strands that provides advantages compared to dideoxy fingerprinting and DNA sequencing. We evaluated the sensitivity and accuracy of GVS by: 1) genotyping separate and pooled DNAs in ratios ranging from 1:1 to 1:40 from a set of 4 plasmids identical except for their cloning sites, 2) determining the allele frequencies from pooled plasmid DNAs mixed in different relative ratios, and 3) genotyping pooled genomic DNAs of 30 individuals with idiopathic hypogonadotropic hypogonadism and 17 controls to detect variations in the GnRH gene. Using GVS we easily identified all plasmid variants when DNAs were screened separately or mixed in ratios from 1:1 to 1:40. We also identified and determined the frequencies of a single base insertion (difficult to identify by automated sequencing), as well as missense and silent substitutions all confirmed by sequencing in 30 non-related IHH probands versus controls. Our data show that GVS can provide an alternative that is more sensitive, efficient and rapid than automated DNA sequencing to detect variations in nucleic acid sequences from populations. Importantly, GVS provides advantages over DNA sequencing in its 1) simplicity of data presentation and analysis, 2) detection of heterozygotes and 3) ability to analyze pooled samples which can reduce the cost to a fraction of that of sequencing.

Identification of Novel alpha-Galactosidase A Mutations causing Fabry Disease. *J. Shabbeer, G. Ashley, C. Eng, M. Yasuda, R. Desnick.* Human Genetics, Mount Sinai School of Medicine, New York, NY.

Fabry disease, an X-linked inborn error of glycosphingolipid catabolism, results from the deficient activity of the lysosomal exoglycohydrolase, alpha-galactosidase A (EC 3.2.1.22; alpha-Gal A). The nature of the molecular lesions in the alpha-Gal A gene in 40 unrelated families was determined in order to provide precise heterozygote detection, prenatal diagnosis, and to explore possible genotype/phenotype correlations. Genomic DNA was isolated from unrelated affected males, and the entire alpha-Gal A coding region and flanking intronic sequences were analyzed by PCR amplification and automated sequencing. Twenty new mutations were identified, including 11 missense and two nonsense mutations, five small deletions, and three small insertions. In the remaining 19 unrelated Fabry families, 16 previously reported mutations were detected. These studies further define the heterogeneity of mutations in the a-Gal A gene causing Fabry disease, and permit precise heterozygote detection and prenatal diagnosis.

Transcript profiling of human endothelial cells defines novel mechanisms by which recombinant human activated protein c regulates pro-inflammatory pathways. *L.M. Gelbert, D.E. Joyce, A.V. Ciaccia, B.D. DeHoff, B.W. Grinnell.* Lilly Research Laboratories, Indianapolis, IN.

Sepsis is an systemic infection-induced inflammation syndrome resulting in septic shock, organ failure, pulmonary dysfunction, cardiovascular failure, renal dysfunction, acquired protein C deficiency, abnormal thrombosis and death. Protein C is a natural anti-thrombotic and plays a key regulatory role in maintaining normal hemostatis. Following activation by thrombin/ thrombomodulin complex on the endothelial surface, activated Protein C (APC) prevents thrombin generation, promotes fibrinolysis, and manifests anti-inflammatory effects. Because of it's thrombotic, fibrinolytic and anti-inflammatory activities, recombinant human aPC (rhAPC) is currently in phase III clinical trials as a treatment for severe sepsis.

While it's thrombotic and fibrinolytic activities are well defined, little is known of the molecular mechanism of how rhAPC modulates the inflammatory cascade. To better understand the mechanism of action of rhAPC in sepsis, we have performed transcript profiling. Human umbilical vein endothelial cells (HUVEC) were treated with TNF-a (a pro-inflammatory cytokine), rhAPC, or a combination of rhAPC and TNF-a. Message RNA from the treated cells was then analyzed using Affymetrix oligonucleotide arrays containing approximately 7000 human genes. Reproducible changes in gene expression were observed for 110 genes in response to one or more treatments. Prominent in the expression profile of aPC was the suppression of a cluster of pro-inflammatory genes. rhAPC inhibited the expression of the NFkB pathway, and down-regulated the adhesion molecules ICAM-1, E-selectin, Fractalkine, and VCAM-1. The down regulation of adhesion molecule gene expression by aPC was further confirmed with Western blot and flow cytometry, and was shown to be dose-dependent.

A physical and transcription map of the candidate region 17q23-q24 for a gene causing Russell-Silver syndrome (RSS). *S. Doerr*¹, *A.T. Midro*², *J. Giannakudis*¹, *I. Hansmann*¹. 1) Inst. f. Humangenetik, Halle, Germany; 2) Dept. of Clin. Genetics, Bialystok, Poland.

Russell-Silver syndrome is mainly characterized by pre- and postnatal growth retardation and dysmorphic features including lateral asymmetry, a small triangular face with prominent forehead and clinodactyly of the fifth fingers. The genetic etiology of the syndrome is unknown and seems to be heterogeneous. Most cases are sporadic although in a minority familial occurrence has been described. About 10% of patients show maternal uniparental disomy of chromosome 7. Furthermore, two autosomal translocations involving band 17q25 were reported in association with RSS (Ramirez-Dueñas et. al., 1992; Midro et. al., 1993). Molecular analysis of the breakpoint on chromosome 17 of the de novo translocation previously described as t(1;17)(q31;q25) enabled us to refine the localization of the breakpoint to 17q23-q24. In order to identify a gene for RSS in the region 17q23-q24 a YAC/PAC/cosmid contig (~ 5 Mb) for the RSS critical region around the breakpoint was constructed. This contig comprises loci for 75 STSs including 34 loci for genes / ESTs. Evidence is provided for a duplication/amplification of 9 loci on both sides of the translocation breakpoint. By sequence analysis the duplicated sequences share identity between 85% and 98%, respectively. This observation has to be taken into account as a possible explanation for the generalogy of the disease: e.g. this region may be target by unequal crossing over between the duplicated regions on both sides of the breakpoint. By searching for expressed sequences within the breakpoint area we identified karyopherin alpha 2 (KPNA2) a gene of a multigene family in close proximity to the breakpoint. We determined the exon-intron structure of this gene and screened 30 RSS-patients for sequence variants by direct sequencing of the 11 exons of this gene and adjacent intron sequences. However, no disease related mutations were detected so far. Interestingly we identified a specific haplotype comprising 6 intragenic polymorphisms showing association to RSS.

An integrated transcript map of the chromosome 13q32 region linked to bipolar disorder and schizophrenia. *S.L. Christian, V. Vlamakis, C.Y. Liu, J.A. Badner, E.S. Gershon.* Dept Psychiatry, Univ Chicago, Chicago, IL.

The identification of susceptibility genes for complex disorders including psychiatric disease has been difficult due to multiple factors including genetic heterogeneity and probable multigenic inheritance of several small effect genes. Whole genome linkage analyses have identified several regions with evidence of linkage to bipolar disorder including 13q32. Additional fine mapping using a total of 13 microsatellite markers from 13q32 were analyzed and confirmed the previous linkage with a peak LOD of 3.7 around D13S779-D13S225. The region of interest is now defined as a ~9 cM region located between 76-85.2 cM on the genetic map. Interestingly, this same region also shows linkage to schizophrenia. To begin molecular characterization of this region, an ~10 Mb physical YAC/BAC contig was developed using mapping information acquired from the Sanger Centre chromosome 13 database and other whole genome databases. This initial map contained 48 microsatellite markers, 106 ESTs and 314 other STSs. The genomic clones identified included 200 YACs and 758 BACs. To confirm the accuracy of the map, a minimal tiling path comprised of 17 YACs and 160 BACs was used for PCR-based STS mapping of all ESTs, genetic markers and the 19 known genes for this region. Additionally, as draft genomic sequence for this region has become available, electronic PCR was used to integrate this data. As a means of confirming the accuracy of the genomic sequence and accelerate high density SNP development of coding regions, 70 unique cDNA clones were sequenced. The physical map and cDNA sequences will now be used for SNP development across individual genes and association studies to identify the susceptibility gene(s) for bipolar disorder.

A large insert clone-based transcript map of human chromosome region 9p13. *C.L. Jackson¹, D.R. Mills¹, S.J. Humphray², R.M. Preparata¹, C. Huang¹.* 1) Department of Pathology, Rhode Island Hospital, Brown University, Providence, RI; 2) The Sanger centre, Wellcome Trust Genome Campus, Hinxton, CB10 1SA, Cambridge, UK.

The short arm of chromosome 9 has become recognized as a region of considerable biological significance to which several disease genes have been mapped by genetic analysis. The gene for cartilagehair hypoplasia (CHH) has been localized previously by linkage analysis to the 9p13 region and is currently believed to fall between the sequence-tagged site (STS) markers D9S165 (distal) and D9S50 (proximal). Similarly, the gene for acromesomelic dysplasia Marteaux type (AMDM) has been recently mapped to a 6.9-cM candidate interval that spans the 9p13-9q12 region of chromosome 9. Briefly, CHH and AMDM belong to a group of diseases classified as heritable autosomal recessive osteochondrodysplasias. Multiple studies using deletion and LOH analyses also suggest that several candidate tumor suppressor genes, in addition to the well characterized INK4a-ARF locus, may reside in this region of the genome. We are in the process of completing a physical map of human chromosome 9p13 that bridges the markers D9S104 (tel) and WI17743 (cen). The contig consists of 55 YAC, 45 BAC, and 4 P1 clones ordered against 116 molecular markers that span an estimated 5 megabases of genomic sequence. The markers are comprised of 44 sequence-tagged sites (STSs), 65 expressed sequence tags (ESTs) and 7 known genes including PAX5, CNTFR, GALT, and the candidate tumor suppressor gene APAH1. Several AluPCR derived polymorphic markers have also been identified that may assist in further genetic refinement of mapped disease loci and for integrating the existing physical and genetic linkage maps of this region. We are currently completing a sequence ready BAC contig of 2X coverage that will accelerate gene discovery efforts from this increasingly interesting region of the human genome.

Physical Mapping of Amish Microcephaly on 17q. *M.J. Rosenberg¹, R.I. Kelley², J. Davis¹, L.G. Biesecker¹.* 1) National Human Genome Research Institute, NIH, Bethesda, MD; 2) 2 Kennedy-Krieger Inst. and Dept. of Pediatrics, Johns Hopkins University, Baltimore MD.

Amish microcephaly is a distinct syndrome with severe congenital microcephaly, hypertonia, irritability, and premature death, usually at six to 15 months of age. In addition, there is increased urinary excretion of 2-ketoglutarate, citrate and aconitate and decreased levels of succinate and fumarate in these patients. The disorder is inherited in an autosomal recessive pattern and is only known to occur in Old Order Amish who have ancestors in Lancaster County, Pennsylvania. Previously, we reported linkage of the disorder to markers D17S1301 and D17S1603 located on the distal portion of chromosome 17q. We have since acquired additional samples from the pedigree and refined the candidate region to lie between markers D17S968 and a new marker that we developed (5837.1) that is located between D17S801 and D17S722. We have constructed a physical map consisting of BAC and PAC clones that encompasses this region. Currently, the map consists of two contigs. We are using STS mapping and BLAST analysis of sequenced BACs to identify new clones to connect the contigs. Search of the high-throughput genome sequence database has identified partially sequenced BAC clones that cover the majority of this region. The current candidate region is estimated to be 3 cM and at least 1 Mb in size. We have begun analysis of these sequences to identify transcripts in the candidate region and have started to sequence the most promising candidates to look for mutations in affected individuals.

A chromosome 21 cSNP map and database. *S. Deustch¹, C. Iseli², P. Bucher², S.E. Antonarakis¹, H.S. Scott¹.* 1) Medical Genetics, Geneva Univ Medical School, Geneva, Geneva, Switzerland; 2) Swiss Bioinformatics Institute, Epalinges, Switzerland.

Single nucleotide polymorphisms (SNPs) are likely to contribute to the study of complex genetic diseases. The genomic sequence of human chromosome 21q was recently completed (33.6 Mb) with 225 annotated genes. This allows efficient identification and precise mapping of potential cSNPs by bioinformatics approaches. Here we present a human chromosome 21 (HC21) cSNP database and the first comprehensive chromosome specific cSNP map. Potential cSNPs were generated using three approaches: i) Alignment of the complete HC21 genomic sequence to cognate ESTs and mRNAs: candidate cSNPs were automatically extracted using a novel program for context dependent SNP identification that efficiently discriminates between true variation, poor quality sequencing and paralogous gene alignments. ii) Multiple alignment of all known HC21 genes to all other human database entries. iii) Gene targeted cSNP discovery. To date we have identified 374 cSNPs averaging approximately 1 SNP per 1.5 Kb of transcribed sequence, covering 64% of known genes in the chromosome. The density of SNPs is highest towards the gene rich telomeric region. Validation of our bioinformatics approach was demonstrated by a confirmation rate of 78% for the predicted cSNPS, and in total 32% of cSNPs in our database have been confirmed. The database is publicly available at <http://cnp.unige.ch> or <http://cnp.isb-sib.ch>. These SNPs provide a tool to study the contribution of HC21 loci to complex diseases such as bipolar affective disorder and allele specific contributions to Down syndrome phenotypes.

The MegaSNPatron: An Ultra-High Throughput Genotyping Platform. *C.A Gelfand, R.A. Swenson, J. Tarantino, B. Hoghooghi, M. Pohl, F. Shemansky, G. Schnerr, M. Boyce-Jacino.* Genomics R&D, Orchid Biosciences, Princeton, NJ.

ABSTRACT The availability of the completed human genome and potentially over 1,000,000 SNP's by the end of 2000 has fueled the demand for rapid, low-cost and flexible methods for single nucleotide polymorphism (SNP) genotyping. There is an emerging opportunity to understand how drug response, adverse drug effects, and genetic predisposition to disease of individual patients can be correlated to specific SNPs on a genome-wide scale. Finding these correlations requires that an ever increasing number of SNP's be analyzed against a very large number of patient samples, and has driven a requirement toward vast genotyping capacity, at least million SNP-samples per day, in order to make these key pharmacogenetic correlations. Orchid has been a leader in high throughput genotyping with its SNPstream 25K, a robotic platform performing Orchids SNP-IT primer extension biochemistry. The next generation technology, internally referred to as the MegaSNPatron, is designed as a series of modules, each capable of routine 100K SNP/day operation with the ability to perform at higher throughputs. To achieve the ultra-high throughputs of the MegaSNPatron, the core primer extension genotyping biochemistry, SNP-IT, has been adapted to a novel fluidics device which draws from the inherent scalability and compatibility with automation of the standard microtiter plate format. Direct fluorescence read-out in a novel array-reading detection system, and PCR and genotyping multiplexing in an array-based platform maintain the processing flexibility features of the SNPstream system while enabling significant per analysis reagent reduction. Results from performance of the MegaSNPatron method for a range of different SNP's will be provided.

The SNPcode 100 Genotyping Kit: A Generic System for SNP Genotyping on High Density Arrays. *M.A.*

Donaldson¹, J. Lathrop², C.A. Gelfand¹, N. Nouri², T. Ryder², M. Boyce-Jacino¹. 1) Genomics R&D, Orchid Biosciences, Princeton, NJ; 2) Affymetrix, 3380 Central Expressway, Santa Clara, CA 95051.

ABSTRACT As the human genome project nears completion, a new, substantial need to obtain rapid, low cost information on genetic variations has emerged. A large pharmacogenetics focus for use of this wealth of genetic information concerns detection of single nucleotide polymorphisms (SNPs), the identity of which are being shown in ever increasing numbers to correlate to medically relevant conditions, such as adverse drug response or predisposition to disease. However, in order to gain insight to these tremendously valuable correlations between SNPs and phenotypes, high-throughput SNP detection systems are required. Orchid BioSciences and Affymetrix have formed a collaboration to develop the SNPcodes 100 kit, combining Orchids SNP-IT primer extension genotyping assay with the uniquely flexible GenFlex Tag Array chip from Affymetrix. The result is a versatile genotyping system capable of determining from 100, in the first kit form, to 2000 genotypes on a single chip, with the ability for the user to adapt any SNP loci of interest to this generic platform. We will present use of this new genotyping system on a number of different SNPs and samples, and the accuracy of the results compared to other genotyping methods.

Bead-Based Determination of Single Nucleotide Polymorphisms by Primer Extension. *M.A Kunkel¹, C.A Gelfand¹, J. Jacobson², M. Boyce-Jacino¹.* 1) Genomics R&D, Orchid BioSciences, Inc., Princeton, NJ; 2) Luminex Corporation, Austin, TX.

Bead-based Determination of Single Nucleotide Polymorphisms by Primer Extension Mark Allen Kunkel¹, Craig A. Gelfand¹, James Jacobson² and Michael Boyce-Jacino¹ 1Orchid BioSciences, Inc. 303 College Road East, Princeton, NJ 08540 and 2Luminex Corporation, Austin, TX ABSTRACT The accelerating interest in SNP genotyping, and the sweeping pharmacogenetic implications of correlating SNPs to phenotypes, has resulting in the need for low cost, high throughput, highly accurate SNP genotyping methods. Orchid BioSciences and Luminex have entered into a collaboration to adapt Orchids SNP-IT primer extension genotyping assay to Luminexs novel bead-based assay platform, capable of rapid, solution-phase assay multiplexing. The result is a low cost, high accuracy SNP genotyping system capable of high-throughput, while maintaining a flexibility to adapt any SNP locus of interest to the assay. The genotyping system is based on the use of Xmm beads labeled with a mixture of two fluorescent dyes. The exact ratio of the two dyes, read by a two laser system in a flow cytometry format identifies a specific bead as unique. The ability to vary the ratio of the two dyes enables hundreds and potentially thousands of unique bead fluorescent signatures to be read from one sample. To use the system for genotyping, unique oligonucleotides are attached to each uniquely labeled bead, creating a substrate for direct genotyping (by primer extension of the oligo attached to the bead) or affinity capture of a complimentary labeled genotyping product. The unique features of this system potentially enable the rapid reconfiguration of the set of SNPs to be tested for any given sample both in terms of which specific SNPs are to be genotyped as well as the multiplexing density of the system. The relative advantages of this bead-based system will be described, along with genotyping results achieved with the system.

Program Nr: 1499 from the 2000 ASHG Annual Meeting

Primer Design for High Throughput Primer Extension. *M.G. Pohl, L. Picoult-Newberg, R. Patch, M. Boyce-Jacino.* Informatics, Orchid BioSciences, Princeton, NJ.

The emergence of single nucleotide polymorphisms (SNPs) as the key measure of genetic diversity in human and other populations and the exploding availability of well characterized SNPs for genotyping, due to the efforts of the SNP Consortium and others, now requires a technique for high volume primer design. A software program and database has been developed for the high volume design of three primers, two for PCR and one for primer extension for each SNP. This program can design primers for 10,000 SNPs per day. It is optimized to incorporate as much information about each sequence as possible, incorporating information about sequence quality, sequence ambiguity, repeat regions and insertion/deletions. The program also incorporates Orchid's algorithm for optimizing efficiency of primer extension which dramatically reduces noise sometimes found with extension primers as well as our algorithm for generating primer extension primers to genotype insertion/deletions. This software was developed for use on the SNP Consortium data set and was an essential feature of actually performing assays on thousands of SNPs. Results are presented on high volume primer design on 10,000 sequences with PCR and genotype success rates.

Polymerase Signaling Assay for DNA Sequencing and DNA Polymorphism Analysis. *R.E Swenson, Q. Xu, C. Gelfand, M. Donaldson, M. Boyce-Jacino.* Applications Development, Orchid Biosciences, Princeton, NJ.

ABSTRACT Due to the rapidly increasing volume of gene sequence data from humans and other species of scientific and clinical interest, there is a long term need for genetic analysis methods which are cost effective but enable a wide range of analyses per sample. Ideally, such technologies inherently are standardizable and generic in that they can be applied with relatively equal likelihood of success to a wide variety (i.e. millions) of gene targets. The challenge we defined, therefore, is to develop a simple system universally suitable for rapid analysis of complex genetic loci, such as genes containing multiple SNPs, and for various diagnostic applications, such as human cancer and infectious disease diagnoses. To this end, we have developed the Polymerase Signaling Assay (PSA), a generic array strategy for generating sequence-specific information from a complete standard and generic set of short oligonucleotides. The Polymerase Signaling Assay is based on capture of the template target, resulting in tethering of the template in the immediate vicinity of the specific portion of the arrayed 4-mers or 5-mers, followed by performing polymerase extension with terminating nucleotide triphosphates. This Orchid universal processor has arrays of a complete set of DNA probes, providing the distinct advantage of being able to sequence or detect variations in any DNA source, in effect sequence-independent resequencing. The core technologies, capture of the template and scanning of the template by the probe/polymerase complex for regions of complementarity, have been developed, in conjunction with the detailed studies of biochemical aspects of the process. Utilizing a simple polyA/polyT capture sequence, we were able to obtain strong signals for synthetic templates with short oligonucleotide arrays. Meanwhile, individual array elements were optimized, along with further improvement of enzyme activity. For the final device, the universal arrays will be fully integrated into a portable, low-cost and universally applicable platform for DNA sequencing, re-sequencing and genotyping applications.

DNA microarrays as a method to monitor changes in mitochondria-related gene expression in mouse mutant cell lines. *S.E. Levy, D.C. Wallace.* Center for Molecular Medicine, Emory Univ, Atlanta, GA.

Mitochondria produce most of the cellular ATP by the process of oxidative phosphorylation (OXPHOS) and generate most of the endogenous oxygen radicals as a toxic by-product. In addition, mitochondria are central in the regulation of apoptosis, calcium homeostasis and cytoplasmic redox state. The 16 kb mitochondrial DNA (mtDNA) encodes only 13 of the proteins involved in OXPHOS. The vast majority of mitochondrial proteins are coded by the nuclear DNA (nDNA). To better characterize the changes in expression of mtDNA and nDNA genes involved in mitochondrial biology we have developed a mouse mitochondrial DNA microarray (Mitochip) encompassing 453 genes involved in mitochondrial energy metabolism, oxidative stress and apoptosis. Using a standard two-color fluorescent system, we have profiled the changes in gene expression in a cultured mouse cell line which lacks mtDNA (r^0) and in a cell line which harbors a mtDNA mutation that imparts chloramphenicol resistance (CAP^R). The r^0 cell line exhibited the expected deficiency in all mtDNA transcripts and a consistent reduction in nDNA OXPHOS gene expression, aconitase and NRBF1. This contrasted with the increased expression of key glycolytic genes, mitochondrial ribosomal proteins, the LON protease and HSP 84. The CAP^R mouse cell line showed a coordinate up-regulation of all mtDNA transcripts, but the down-regulation of multiple nDNA OXPHOS genes. Profiling of a mouse fibroblast cell line grown in media supplemented with glucose, pyruvate and uridine revealed a down-regulation of the LON protease and HSP 84, opposite to that observed in the r^0 cell line. These data demonstrate that even relatively minor changes in mtDNA function can have profound effects on the mitochondrial gene expression profile. Thus, mitochondrial gene expression profiling will be a valuable aid in the analysis of model systems for human mitochondrial disease.

Discovery and analysis of recent and ancient polymorphic human L1 insertions. *I. Ovchinnikov¹, A. Troxel², G. Swergold¹.* 1) Dept. Medicine, Columbia University, New York, NY; 2) School Public Health, Columbia University, New York, NY.

The retrotransposition of LINE-1 elements has played a major role in shaping mammalian genomes. When a new L1 insertion occurs, a stably inherited identical-by-descent genetic locus is created. These LINE Insertion Dimorphisms or LIDs, are valuable tools for human genomic and evolutionary studies. We developed a novel PCR-based method called LINE-1 display to enable the large-scale discovery of LIDs. A major advantage of LINE-1 display over previous methods is its ability to discover both recent and ancient L1Hs insertion events that are expected to have low and high gene frequencies respectively. In this study we detected a total of 152 polymorphic bands in 91 DNA samples representing many worldwide human populations. Each LINE-1 display band contains the 3' terminus of an L1 insertion, a poly-A tail, and a variable length 3' flanking sequence. We analyzed 53 unique polymorphic insertions belonging to the L1Hs-Ta class that is currently undergoing amplification in the human population. BLAST searches identified 35 of the LID loci. No L1 insertion was present at 23/35 Genbank loci suggesting that many of these LIDs had low gene frequencies. We selected 30 non-Ta, primate-specific L1Hs insertions from the human Genbank database for comparison with the 53 LIDs. Our analyses revealed that LIDs had significantly longer poly-A tails (13.9 vs 3.7, $p=0.0001$), fewer polymorphic L1Hs nucleotides (12 vs 17, $p=0.004$), and fewer poly-A associated microsatellites (3 vs 9, $p=0.002$) confirming that they represented a younger population of insertions. In contrast, no significant differences were found in the presence of various other types of genomic repeats in the flanking sequences of the 2 groups. Interestingly, the LID 3' flanking sequences had significantly higher GC contents, and were distributed randomly among genomic isochores. These data suggest that L1Hs transpositions insert into random genomic loci and that the nonrandom distribution of L1s in the modern human genome is a result of events that occur after transposition.

X chromosome-specific cDNA arrays: large-scale identification of genes that escape from X-inactivation and other applications. *U.A. Nuber¹, R. Sudbrak¹, G. Wieczorek¹, W. Mann¹, R. Kirchner¹, C.J. Brown², D. Woehrle³, P. Sterk⁴, V. Kalscheuer¹, W. Berger¹, H. Lehrach¹, H.H. Ropers¹.* 1) MPI for Molecular Genetics, Berlin, Germany; 2) Department of Medical Genetics, University of British Columbia, Vancouver, Canada; 3) Department of Medical Genetics, University of Ulm, Germany; 4) European Bioinformatics Institute, Hinxton, Cambridge, United Kingdom.

Since their first description less than 5 years ago, cDNA microarrays have become indispensable tools for monitoring gene expression profiles in a wide variety of species and tissues. In cultured cells, expression profiling with cDNA arrays has led to the identification of genes that are regulated in a coordinated manner during the cell cycle, under nutritional constraints or after treatment with hormones. cDNA based gene expression profiling in normal and malignant tissues is beginning to shed light on the molecular mechanisms involved in tumorigenesis and tumor progression. Similar approaches are being employed in cell culture or animal models for drug testing, and another emerging field is the search for secondary effects of defined gene mutations on the gene expression profile to identify downstream target genes and to elucidate the pathogenesis of hereditary disorders. In contrast, cDNA array-based expression profiling has not been employed yet to search for primary defects underlying genetic disorders. To study the potential of this approach, we have generated a microarray carrying 2423 cloned cDNA fragments which represent up to 1317 different X-chromosomal genes. As a prelude to testing cell lines from patients with X-linked disorders, this array was used to compare gene expression profiles in lymphoblastoid cell lines from healthy males and females. These studies, and subsequent experiments with cell lines from probands carrying supernumerary X chromosomes, have enabled us to identify numerous new genes which escape from X-inactivation. Moreover, we show here that chromosome-specific cDNA arrays are powerful novel tools for identifying disease genes.

Expressed single nucleotide polymorphisms (cSNPs) as markers of allele specific gene activity in the X chromosome. *L.R. Vasques, S.J. Kim, L.V. Pereira.* Depto. Biologia, IB, Universidade de Sao Paulo, SP, Brazil.

Single nucleotide polymorphisms (SNPs) are the most common sequence variations found throughout the human genome, occurring with a frequency of 1 in 100-300 bases. As of May, 2000, the SNP database (dbSNP) at The National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/SNP/index.html>) had collected more than 135,000 of these markers. Although most SNPs lie in non-coding regions of the genome, a fraction is located in expressed sequences (cSNPs). These can be used as markers to study allele specific gene expression, particularly of imprinted genes and, as proposed here, of X-linked genes. We have sequentially analyzed the data on each of the 423 X-linked SNP described in the dbSNP at NCBI, identifying 125 which are located in expressed sequences. Twenty-two of these markers were used in a pilot study of the application of cSNPs to monitor X-linked gene activity in primary normal human cells. The corresponding ESTs/cDNAs were RT-PCR amplified from fibroblast, lymphocyte and chorionic villi RNA, establishing a tissue specific profile of expressed SNP-containing-ESTs (SNP-ESTs). Allele specific expression of SNP-ESTs was investigated in 4 independent primary normal human fibroblasts with completely skewed X inactivation patterns. Genotyping was performed by amplification of genomic DNA and direct sequencing of PCR products. Collectively, 11 cSNPs were informative. Allele specific expression of each one was verified by RT-PCR and direct sequencing. Until now, the most extensively used system for studying gene expression from the inactive X has been rodent/human somatic cell hybrids. However, due to the unnatural characteristic of the hybrid cells, one cannot ensure that they faithfully reproduce normal control of gene activity. Based on our results, we propose that X-linked gene expression should be studied in normal human cells using cSNPs. A similar approach can be extended to the analysis of autosomal cSNPs for the identification of imprinted genes. This system may reproduce more precisely allele specific gene expression in humans. FUNDING: FAPESP, CNPq.

A Novel Congenital Autosomal Dominant Hyaline Body Myopathy in a Saudi Family Showing Linkage to Chromosome 6q. *S.N. ABU-AMERO, B.F. MEYER, C.E. BOUMAH, C. HODGKINSON, V. BARUCHA, A. BUTT, E. CUPLER, M. KAMBOURIS, S. BOHLEGA.* GENOMICS, KFSH&RC, RIYADH, SAUDI ARABIA.

We describe a novel congenital hyaline body myopathy (HBM) showing autosomal dominant (AD) inheritance in a 3-generation consanguineous Saudi family, with 8 of 13 children, mother and maternal grandfather affected. There is slow, progressive weakening of muscle and atrophy develops with scapuloperoneal distribution. In 6 of the 9 patients presentation was non-progressive proximal weakness with no bulbar, facial or ocular weakness although with significant wasting and loss of subcutaneous fat. Creatine phosphate was mildly elevated (2-5 times normal). Two of the affected members of this family showed progressive scapuloperoneal weakness with loss of ambulation by the age of 14. Muscle biopsy show discrete subsarcolemmal hyaline bodies in approximately 20% of type 1 skeletal muscle fibres. They consist of granules which may be in linear array, filaments or amorphous components, with the rest of the sarcomeric organization intact. They stain pale pink with hematoxylin-eosin and pale green by the modified Gomori trichrome stains. The hyaline bodies display myofibrillar ATPase activity following acid pre-incubation. Evidence suggests that hyaline bodies are the result of myosin heavy chain breakdown. AD HBM presents as scapuloperoneal syndrome (SP) that has been mapped to 12q (D12S82-D12S101). The neurogenic form of SP has also been mapped to a 19cM interval on 12q24.1-q24.31, 7-38cM from the myopathic form suggesting that this region on 12q may harbour a family of genes involved in SP pathology. Using a subset of these and other markers in this region, 12q was excluded as harbouring the gene underlying AD HBM in our family, pointing to a novel gene responsible for this myopathy. A whole genome scan was undertaken using the CHLC marker set and results suggested linkage to 6q (D6S1056 and D6S474). A further 27 markers were used to establish linkage. Based on this data the gene causing AD HBM in this family lies within a 7cM region between D6S1596 and D6S1625 as defined by recombination events in affected and unaffected members of the pedigree.

Screening of SLC1A1 for mutations in an American population of cystinurics. *W.L. Gitomer^{1,2}, B.Y. Reed^{1,2}, C.Y.C. Pak¹*. 1) Center for Mineral Metabolism , UT Southwestern Medical Ctr, Dallas, TX; 2) Internal Medicine, UT Southwestern Medical Ctr, Dallas, TX.

Cystinuria is a renal transport disorder characterized by excess excretion of cystine and dibasic amino acids (DAAs) and cystine kidney stone formation. Cystinuria is phenotypically classified into three subgroups and has been shown to be due to mutations in SLC3A1 (Type I) and/or SLC7A9 (Types II & III). These genes code for subunits of a low Km DAA transporter. However, not all cystinurics have been shown to have mutations in either of these two genes. The low Km transporter is one of two renal cystine transporters. The other transporter has a high Km for cystine and is specific for cystine. Since only the low Km transporter also transports DAAs, this is the transporter thought to be involved in cystinuria. However, a decrease in the activity of the high Km transporter would cause increased luminal cystine, this then could act as a competitive substrate for the low Km transporter resulting in the inhibition of DAA resorption and increased urinary excretion. Recently Low et al have shown that the glutamate/aspartate transporter, which is encoded for by the SLC1A1 gene, has similar cystine uptake kinetics and renal tubule localization as the high Km cystine transporter. To determine if this gene is involved in cystinuria, we are screening 52 unrelated cystinurics for mutations in genomic DNA of the entire coding region of the SLC1A1 gene (12 exons) and the intron-exon boundaries. Initial screening has revealed 9 different base changes to date: A232+14T, C233-75T, C233-28T, C233-23T, A325+55G, G414A, A875+8C, C1110T, and G1193+11A. Neither the G414A mutation (codon acg to aca) nor the C1110T mutation (codon acc to act) result in a change in amino acid. Screening of the rest of the gene for mutations and the determination if any of the intronic base changes effect RNA splicing will be required to determine if the SLC1A1 gene product is involved in the etiology of cystinuria. Low, RK, Schreiber, CK, Iwahashi, CK, Abalos, JJ, Williams, HE (2000) Urolithiasis 2000, 9th International Symposium on Urolithiasis, Abstracts p 79.

CYSTINURIA TYPE I, GROWTH AND MENTAL RETARDATION, AND DYSMORPHISM ASSOCIATED WITH SLC3A1 GENE DELETION . *I. Brodyansky¹, E. HersHKovitz¹, R. Parvari²*. 1) Dept. of Pediatrics, Soroka Medical Center, Beer Sheva, Israel; 2) Department of Immunology and Microbiology, Ben Gurion University, Beer-Sheva, Israel.

Cystinuria is a heritable disorder of cystine and dibasic amino acids transport. We describe six patients with a unique clinical picture and deletion of all exons of the SLC3A1 gene (Solute carrier family 3, member 1) which encodes the carrier transporter of dibasic amino acids and cystine. The pattern of inheritance is autosomal recessive. The patients were diagnosed during the first year of their life with facial dysmorphism, neonatal seizures, hypotonia, and severe somatic and developmental delay. Renal stones were found in 4 patients. The major laboratory findings were: neonatal hypocalcemia and hypoglycemia and lactic acidosis (3 patients). Urinary cystine, lysine, arginine and ornithine, levels were compatible with its level in cystinuric homozygotes patients. The parents and all the apparently healthy siblings had normal urinary excretion profile of amino acids, establishing the diagnosis of cystinuria type I. Muscle biopsy in one patient showed red ragged fibers and low activity of complex I, IV, and V, with normal complex II and PDHc activities. Linkage analysis by homozygosity disclosed a linkage to D2S119 and D2S177 close to the locus of the SLC3A1 gene. The 10 SLC3A1 exons could not be amplified from genomic DNA by PCR. DNA samples from the parents yielded normal amplifications of the SLC3A1 gene exons. Since cystinuria is probably not associated with mental retardation and as no clinical segregation was found among the rest of the family members, we suggest that this unique clinical syndrome is most likely a result of a deletion of several genes that include the SLC3A1 locus.

Gene redundancy in patients with the hyperornithinemia-hyperammonemia-homocitrullinemia (HHH)

syndrome. *J. Camacho*^{1,2}, *C. Obbie*¹, *J. Porter*², *J. Kong*², *D. Valle*¹. 1) Pediatrics/Medical Genetics, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Human Genetics, Oklahoma Univ Health Sciences Center, OKC, OK.

The mitochondrial ornithine transporter ORNT1, a member of the mitochondrial carrier family (MCF) of proteins is the gene defective in patients with the HHH syndrome. Patients with HHH syndrome have a later onset presentation and less severe phenotype when compared to other urea cycle disorders. As an example a group of French Canadian patients with the F188 D mutation (which produces an unstable protein) have a wide range of phenotypes and manage to remain in good control in a restricted protein diet and citrulline. These observation suggest that ornithine is transported to the mitochondria by other MCF proteins, a possible gene redundancy effect. We discovered an intronless gene termed ORNT2 (5q31) which is 88% identical to ORNT1. Northern analysis demonstrated that ORNT2 is mainly expressed in the liver, kidney and pancreas, while ORNT1 is also expressed in the liver and pancreas but not in the kidney. Utilizing gene specific primers, RT-PCR experiments demonstrated that poly A+ RNA derived from controls and HHH-F188 D patients also expressed ORNT2. Utilizing N-myc tagged constructs it was demonstrated that ORNT2 has a cellular targeting pattern of mitochondrial proteins similar to ORNT1. When wild type and N-myc tagged ORNT2 were overexpressed in cultured HHH fibroblast it restored ornithine metabolism similar to ORNT1. However it was observed that when ORNT2 was over-expressed in cells carrying the F188 D mutation versus the E180K which produces a stable but non-functional protein, the restoration of ornithine metabolism was significantly higher in the latter. All these observations suggest a potential gene redundancy effect in patients with HHH syndrome and raises the possibility that other members of the ORNT MCF subfamily have ornithine transporting abilities. Secondly, it raises the question why doesnt ORNT2 compensates for a defective ORNT1 in HHH patients, is this due to a gene dosage effect or that ORNT1 and ORNT2 need to heterodimerize as a requirement for maximum ornithine transporting capacity in human mitochondrias.

Reference values of plasma free amino acids in Taiwan Chinese: evaluation of age effect on amino acid analysis.

C.K. Chuang, S.P. Lin. Medical Research Dept., MacKay Memorial Hospital, Taipei, Taiwan.

For an accurate interpretation of free amino acid (AA) results, the application of a well-documented AA reference values is required. Up-to-date, no report states plasma free AA reference values designated in Chinese, and thus, an inappropriate use of AA values from the Western countries became our concern. In this study, the reference values for plasma free AAs in Chinese of 3 age groups (i.e. 0-24 months, n = 24; 2-18 years, n = 38; and adults over 18 years old, n = 46) were determined by executing a high performance AA analyzer. The reference values showed a very good correlation ($r = 0.954$ and $r = 0.975$) to those reported by Scriver CR (Metabolism, 1985) and Shapira E (Biochemical Genetics, 1989) respectively, and indicated plasma AA concentrations were not evidently affected by different human races and dietary convention. We also evaluated and confirmed the age effect on plasma free AA analysis. More than two thirds of a total 35 AAs showed statistically significance (p values < 0.05) based on one way ANOVA test. Amino acids including taurine, hydroxyproline, threonine, serine, glutamic acid, citrulline, and α -amino-h-butyric acid were highly affected by the age effect (p values < 0.01). Phosphoserine, phosphoethanolamine, asparagine, glutamine, valine, methionine, leucine, phenylalanine, ethanolamine, ornithine, lysine, histidine, and arginine showed moderately affected by the age factor (p values < 0.05). Only a few AAs, including α -amino-adipic acid, glycine, alanine, cystine, homocystine, isoleucine, tyrosine, γ -aminobutyric acid and tryptophan, were statistically insignificant in this regard. When comparing the mean difference of individual AA among 3 age groups, alanine, tyrosine, phenylalanine, glycine, glutamine, cystine, homocystine, isoleucine, γ -aminobutyric acid, and tryptophan, did not show any significant differences and appeared to be constant across all age groups. On the contrary, only glutamic acid and serine showed statistically significance (p values < 0.05) and gave a definite decline in concentrations from infancy to adulthood. However, there was no significant correlation obtained from one age group to the others.

Increased prevalence of stimulant use for attentional disorders in children with Phenylketonuria. *G.L. Arnold, C.J. Vladutiu, J. DeLuca, E. Blakely, C. Orlowski.* Dept. Pediatrics, Univ Rochester Sch Medicine, Rochester, NY.

Preliminary evidence suggests attentional dysfunction is more common in children with phenylketonuria (PKU) than in the general population. We reviewed the charts of all children ages 5 - 18 years with early and continuously treated classic PKU followed by the Inherited Metabolic Disorders Clinic at the University of Rochester, in order to determine the prevalence of stimulant use for attentional dysfunction. Use of stimulants was neither prescribed nor suggested by clinic staff. Comparisons between the study group and a control group consisting of age and gender matched children with Type 1 diabetes mellitus were made using t-tests and Chi-square analysis as indicated.

Thirty six children met age requirements for the study. Thirteen of thirty six (36%) were on various stimulant medications. This percentage was significantly higher than the control group ($p < 0.05$) or the average population prevalence of Attention Deficit Hyperactivity Disorder. Stimulant use was also correlated with poor metabolic control. Only 1 of 13 stimulant users maintained phenylalanine levels near or within the suggested treatment range, compared to 14 of 23 non-stimulant users ($p < 0.05$). We report a high prevalence of stimulant use for attentional dysfunction in this group of children with PKU, as well as a strong correlation between stimulant use and poor metabolic control. We speculate that elevated phenylalanine result in CNS dysfunction thereby predisposing these children to attentional abnormalities.

INBORN ERRORS OF METABOLISM OF SAUDI ARABIA. *P. Ozand*¹, *A. Al Aqeel*². 1) Departement of Pediatrics, King Faisal Specialist Hospital, Riyadh, Saudi Arabia; 2) Departement of Pediatrics, Riyadh Armed Forces Hospital, Riyadh, Saudi Arabia.

Middle Eastern cultures are tribal and heavily consanguineous. Marriage between cousins has been part of the culture for millennia leading to "founder" effect and a large number of autosomal recessive diseases. These ethnic groups did not mix with their neighbors, therefore most of these disorders are unique to the Middle East and are either rare by Western standards or are unknown. A review of the combined files of the Armed Forces Hospital and the King Faisal Specialist Hospital and Research Centre, Riyadh, documented more than 150 varieties of neurodegenerative diseases among 2,000 children; 27 of which constitute more than half of these files. The early recognition of these disorders is important to initiate early treatment especially in cases of organic acidemia and aminoacidemia to prevent neurologic crippling, and in lysosomal storage disorders to initiate early bone marrow transplantation, or enzyme replacement.

Missense mutations in the regulatory domain of cystathionine b-synthase can functionally suppress patient derived mutations in cis. *W.D. Kruger, X. Shan.* Population Sci, Fox Chase Cancer Ctr, Philadelphia, PA.

Human cystathionine beta synthase (CBS) is an S-adenosylmethionine regulated enzyme that plays a key role in the metabolism of homocysteine. Mutations in CBS are known to cause homocystinuria, an inborn error of metabolism characterized by ectopia lentis, osteoporosis, mental retardation, and thrombotic vascular disease. We have previously developed a yeast functional assay for CBS and used it to characterize CBS mutations found in homocystinuric patients. We discovered that most patient derived mutations are functionally suppressed by deletion of the C-terminal 142 amino acids that contains a 53 amino acid motif known as the "CBS-domain". This domain is found in a wide variety of proteins of diverse biological function. We have now used a genetic screen to identify missense mutations in the C-terminal region of CBS that can suppress the most common patient mutation, I278T. Seven suppressor mutations were identified, four of which map to the CBS domain. When combined in cis with another pathogenic mutation, V168M, 6 out of 7 of the suppressor mutations rescued the yeast phenotype. Enzyme activity analysis indicates that the suppressors restore activity from <1% activity to 10% to 60% of the wild-type levels. Analysis of the suppressor mutations in the absence of the pathogenic mutation indicates that most of these suppressor alleles have lost enzymatic responsiveness to S-adenosylmethionine. Our results indicate that subtle changes to the C-terminus of CBS can restore activity to mutant CBS proteins and provide a rationale for a drug screening strategy to search for compounds which can activate mutant CBS alleles.

Distribution of mutations among the three genes causing Maple Syrup Urine Disease. *M.M. Nellis^{1,2}, D.J.*

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Maple Syrup Urine Disease is an autosomal recessive disorder resulting from mutations in any of three genes. These mutations impair the function of branched chain α -ketoacid dehydrogenase (BCKD), a mitochondrial multienzyme complex responsible for the decarboxylation of the ketoacids of leucine, isoleucine, and valine. Buildup of the branched chain amino acids can lead to death in untreated MSUD patients, necessitating newborn screening for the disease. Mutant alleles are distributed among each of the three genes and are unique within families, with most patients being compound heterozygotes. The task of determining specific mutations and establishing a genotype-phenotype relationship is difficult because the defective gene must first be identified. Here we describe a method for restoring BCKD activity to patient derived cell lines using retroviral transduction with cDNA for each of the three gene products (E1a, E1b, E2) to identify the gene harboring the mutant allele(s). Transduction of fifty-five cell lines (25 female, 30 male) showed that 31% have mutations E1a, 36% in E1b, and 24% in E2. Five cell lines gave ambiguous results by complementation with zero or two subunits, and all were from male patients. In females, 44% of the mutations are in E1a, while in males only 20% of the mutations are in E1a ($p < 0.01$). Knowing the affected gene allows nucleic acid sequencing in order to identify specific nucleic acid changes. Inheritance of the identified nucleic acid change is confirmed by analysis of the parental cell lines. The crystal structure for the E1 $\alpha_2\beta_2$ tetramer is known and we are using this to model nonsense mutations within these genes. These studies will define the frequency of gene mutations in MSUD, establish clinical consequences for each gene, and define the functionally important regions of each protein product. Further, the information will establish the feasibility of gene therapy for the different mutations and may lead to the development of specific therapeutic drugs for use in treatment of MSUD without requiring gene therapy.

The effect of maternal hyperphenylalaninemia on the offspring of the BTBR-Pah^{enu2} mouse model for phenylketonuria: A maternal phenylketonuria model. *D. McDonald, S. Cho.* Dept Biological Sci, Wichita State Univ, Wichita, KS.

The BTBR-Pah^{enu2} mouse phenylketonuria (PKU) model and a defined diet (Research Diet #TD97152, Harlan-Teklad, Madison, WI) were used to examine the effect of maternal hyperphenylalaninemia (HPH) on pregnancy outcome. The diet permitted the control of maternal blood phenylalanine (PHE) levels to produce either normal levels or levels of HPH similar to untreated classical human PKU. Pregnancy outcome was examined by measuring three developmental parameters commonly noted as being reduced among the offspring of untreated human PKU females: birth weight (BW), body length measured as crown-to-rump length (CR), and head circumference (HC).

When maternal blood PHE was increased to classical PKU levels during gestation, these pregnancy outcome parameters were all significantly reduced. Furthermore, reductions were seen under two different comparison conditions: 1) when mutant and non-mutant females were maintained on a diet that produced classical PKU levels of maternal HPH during pregnancy (i.e., maternal genotype varied, diet constant); and 2) when mutant females were either exposed to a diet that produced classical PKU levels of maternal HPH or to one that produced normal maternal blood PHE levels (i.e., maternal genotype constant, diet varied). Under the first condition, the observed reductions yielded the following P values: BW, CR, and HC were $P=0.0019$, $P<0.0001$, and $P=0.05$, respectively. Under the second condition, $P<0.0001$ for all pregnancy outcome parameters.

These experiments establish that the BTBR-Pah^{enu2} mouse models some of the most important aspects of human maternal PKU and, thus, should prove useful for intensively investigating important questions relating to this syndrome. By using the above experimental design but producing lower levels of maternal HPH, one can investigate the dose-response relationship between maternal HPH and pregnancy outcome. By short pulses of maternal HPH during pregnancy, one can investigate whether there are sensitive periods during gestation where teratogenic insult leads to specific pregnancy outcomes.

Construction and characterization of a mouse model for human hyperargininemia. *R. Iyer^{1,4}, R. Kern⁴, P. Yoo³, N. Rozengurt¹, W. O'Brien⁵, K. Lu¹, W. Grody^{1,2,4}, S. Cederbaum^{1,3,4}.* 1) Pathology & Lab Med, UCLA, Los Angeles, CA; 2) Pediatrics, UCLA, Los Angeles, CA; 3) Psychiatry, UCLA, Los Angeles, CA; 4) Mental Retardation Research Center, UCLA, Los Angeles, CA; 5) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Deficiency of liver Arginase (AI) causes hyperargininemia, a disorder characterized by progressive mental impairment, growth retardation, spasticity, and punctuated, often fatal episodes of hyperammonemia. We constructed a 'knockout' mouse strain carrying a non-functional AI gene, by homologous recombination. Arginase AI knockout mice completely lack liver arginase (AI) activity, exhibit severe symptoms of hyperammonemia, and die at day p10-14. Unlike human patients where AII activity in kidney is induced 3-34 fold during hyperammonemic episodes, no elevation of AII activity in either kidney or brain was found in these mice, possibly accounting for their more rapid and severe clinical course. During hyperammonemic crises, plasma ammonia levels were increased >10-fold, compared to normal animals. Livers of AI-deficient animals showed hepatocyte abnormalities including cell swelling and inclusions. Plasma amino acid analysis showed the following: Mean arginine levels in knockouts were ~ 4-fold greater than the wildtype, and 3-fold greater than heterozygotes; whereas proline levels were ~1/3, and ornithine levels were 1/2 that of wildtype or heterozygote - predictable biochemical consequences of arginase deficiency. Glutamic acid, citrulline and histidine levels were elevated to about 1.5-fold that seen in the phenotypically normal set. Concentrations of the branched-chain amino acids valine, isoleucine, and leucine were reduced to half normal values. Most of these observations are consistent with liver failure and the consequent perturbation of homeostasis. In summary, the AI-deficient mouse duplicates several pathobiological aspects of the human condition, and should prove to be a useful model for further study of the disease mechanism(s), and to explore treatment options, such as pharmaceutical administration of sodium phenylbutyrate and ornithine, and development of gene therapy protocols.

Inherited metabolic disorders in Taiwan: the MacKay Memorial Hospital experience. *S. Lin, C.K. Chuang.*

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Inherited metabolic disorders (IMDs) are important causes of disease in the pediatric age group. The number of known IMDs has increased tremendously since Garrod first described them in 1908. Many of them can now be treated effectively. Even when treatment is not available, correct diagnosis permits couples to make informed decisions about future offspring. Taiwan is a subtropical island state occupying an area of 360,000 km² and has a population of approximately 22 million. Until 1999, nearly 98% of the population was covered by a national health insurance scheme that included preventive and curative out-patient care as well as hospital care. MacKay Memorial Hospital is a church-affiliated private institution and one of the 11 tertiary medical centers with medical genetics services. Together with 3 other hospitals, it serves the northern part of Taiwan covering a population of 5.5 million. Our hospital also offers special genetic tests to all the hospitals islandwide. Over the past 15 years, a team of clinical and research specialists and associated laboratories for helping managing IMDs has been evolved and become nearly well-established. We also set up close cooperative relationship with genetic departments and laboratories abroad. Up to now, we have been taking care of more than 150 profound IMD patients and their families, including organic acidemias, urea cycle disorders, mucopolysaccharidoses, Gaucher disease, glycogen storage disease, fucosidosis, metal disorders, etc. Patient and family associations are founded, and they exert enormous impacts not only to the IMD families themselves but also to the whole society. Our efforts have helped establish the Taiwan Foundation for Rare Disorders. A new law for the provision of rare disorders patient care with Orphan Drug Act included has been passed. The outlook of clinical services for IMD patients in Taiwan, we consider and do hope, has a cautiously bright future.

Maple syrup urine disease: Variables of treatment. *H. Morton, D. Robinson, A. Haupt, E. Puffenberger, R. Kelley.*
Clinic for Special Children, Strasburg, PA.

We have managed 37 neonates with classical maple syrup urine disease (MSUD) by nutritional methods, without dialysis. Effective management causes plasma leucine concentrations to decrease at higher rates than obtained by dialysis. The acute neurological syndrome of MSUD arises from increased plasma leucine levels which cause reduced uptake of competing neutral amino acids into the brain. Disruptions of tyrosine, tryptophan, and histidine transport result in decreased neurotransmitter synthesis with dystonia and alterations in consciousness, mood, and appetite. Chronically low uptake of essential amino acids such as valine causes delays in myelin synthesis and abnormalities of dendritic branching. A 9-day-old neonate presented with dystonic rigidity, encephalopathy, tense fontanelle, and split skull sutures. Cerumen smelled like maple syrup, urine ketones were large, and urine DNPH reaction was positive. Plasma leucine was 3535 $\mu\text{mol/L}$ (46 mg/dl). Calculated transport rates of neutral amino acids, expressed as % normal, were: Leu 463%, Ile 201%, Val 179%, Phe 10%, Trp 2%, Met 14%, Tyr 9%, and His 18%. Enteral and intravenous nutrition consisted of 195 cal/kg/day (50% as lipid) and amino acids without leucine. Ile 85, Val 113, Gln 411, and Ala 480 mg/kg-24 hrs were given to prevent deficiencies and support protein synthesis. Enteral tyrosine 400 mg/kg-24 hrs increased plasma Tyr levels and transport. Mannitol, furosemide, and hypertonic saline were given to keep serum osmolarity >300 mosm/L and prevent hyponatremia. Plasma leucine decreased from 3535 to 362 $\mu\text{mol/L}$ (46 to 5 mg%) in 48 hrs or 1587 $\mu\text{mol/L/day}$ (-21 mg%/24 hrs). Dystonia resolved as plasma Tyr increased from 47 to 421 $\mu\text{mol/L}$ and the Tyr transport rate rose from 9% to 121%. Plasma Trp and His levels and transport rates remained low, suggesting that more should be added to the formula. Intravenous hyperalimentation was stopped at 36 hrs. On day 3, dietary leucine was added and oral feedings were started. By day 4 her daily oral intake was 100 cal/kg, total protein 2.5 g/kg, Leu 60, Ile 35, and Val 45 mg/kg. On day 5, her fontanelle was soft and all neurological signs of intoxication were gone.

Treatment of neonatal onset Non-Ketotic Hyperglycinemia with combined Sodium Benzoate and

Dextromethorphan regimen. *M.Y. Hasan*¹, *M. Velinov*^{2, 3}, *V. Agarwalla*¹, *G. Kupchik*². 1) Pediatrics, Brookdale University Hospital and Medical Center, Brooklyn, NY; 2) Maimonides Medical Center, Brooklyn, NY; 3) NYS Institute for Basic Research, Staten Island, NY.

Non-Ketotic Hyperglycinemia (NKHG) is an inborn error of metabolism, due to defect in the glycine cleavage system, with excessive accumulation of glycine in the plasma and central nervous system (CNS). It presents with progressive encephalopathy, seizures and hypotonia. Different approaches for the treatment of this condition were previously suggested including low protein intake, administration of Sodium Benzoate, Dextromethorphan, and Imipramine. Finally, dialysis was used in severe cases. We report on a male infant with severe neonatal type of NKHG, who presented at 6 days of age with acute encephalopathy, hypoglycemia, severe dehydration, and respiratory failure. On admission his urinary glycine level was 40989 mmols/l, at 11 days of age his CSF glycine was found to be 264 mmols/l, and plasma glycine 1162mmols/l, with CSF/plasma glycine ratio of 0.23. After establishing the diagnosis of NKHG, treatment was started with the combination of Sodium Benzoate 500mg/kg/day, and Dextromethorphan 5mg/kg/day. The response to this therapeutic regimen was very good. After about 10 days of therapy it became possible to discontinue the respiratory support. 3 weeks after initiation of treatment it became possible to manage this condition on an outpatient basis. At the discharge, glycine levels were 102 and 86 mmols/l in plasma and CSF respectively, with a ratio of 0.84. Sodium Benzoate is used because of its action in reducing the glycine levels in body fluids. Dextromethorphan acts by blocking the NMDA receptors in the brain (Hamosh A - J Pediatr - 1998 Apr; 132(4): 709-13). Our regimen proved to be very effective in counteracting the acute effects of elevated glycine concentration in CNS. Such treatment may be lifesaving in cases of severe neonatal forms of NKHG. Long term follow up with patient is needed in order to assess the consequences for growth and development of this therapeutic approach.

P1173L is a recurrent mutation in the methionine synthase gene causing *cblG* hyperhomocysteinemia. *D.S.*

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Methionine synthase deficiency (*cblG*) is a rare inborn error of vitamin B₁₂ (cobalamin) metabolism that usually presents in infancy or childhood with hyperhomocysteinemia, hypomethioninemia, megaloblastic anemia, and developmental delay. Occasional patients have presented only in adult life. The methionine synthase gene on chromosome 1q43 consists of 33 exons. By homology to methionine synthases from other organisms, binding regions for homocysteine, methyltetrahydrofolate, cobalamin, and S-adenosylmethionine have been determined. We have searched for the causal mutations in genomic DNA from 21 *cblG* patients, representing almost all known patients. Each of the 33 exons of the methionine synthase gene was amplified using flanking intronic oligonucleotide primers. These were analyzed by automated sequencing. Mutations were found in all four binding domains. In addition, the P1173L mutation in exon 31 was found in 10 patients, suggesting that it is a common mutation causing *cblG*.

Methionine Synthase Deficiency Responsive to B12 and Betaine Therapy. *P.W.K. Wong¹, P. Heydemann¹, K. Hyland²*. 1) Pediatrics, Presbyterian St Lukes Med Ctr, Chicago, IL; 2) Institute of Metabolic Disease, Dallas.

Functional methionine synthase deficiency may be divided into 2 groups, cblG and cblE. These patients have megaloblastic anemia. Other patients without megaloblastic anemia have a mutation in the methionine synthase gene. We studied a white male infant, from unrelated parents, who presented with intractable seizures at 2 months of age and had 158 nM/ml homocysteine (N<14nM/ml) and 7nM/ml methionine (N 9 - 65 nM/ml) in the plasma. EEG showed multifocal epileptiform activities and a MRI showed a small area of left extra-cerebral soft tissue density. He had normal CBC without megaloblastic anemia and his urine did not show methylmalonicaciduria. CSF 5-methyltetrahydrofolate was 319nM/L (N=40-170). Lymphocyte methylenetetrahydrofolate reductase activities from the patient and the parents were 3.7, 3.28, 5.52 nM HCHO formed/hr/mg protein (N=10.33) respectively. The parents' enzyme was also thermolabile. Methionine synthase in the skin fibroblasts of the patient and control were 0.06, 0.16 and 3.85, 3.77 nM met/hr/mg protein. Addition of methyl B12 up to 10X the amount in standard culture medium or in the incubation mixture did not significantly increase enzyme activity in patient fibroblasts. A single injection 12mg of 5-methyltetrahydrofolate intraperitoneally and 8mg intravenously was given to different CD1 mice weighing 25 g. No seizure was observed for 1 week. Treatment with 2.5 mg of folic acid 2X daily in the patient reduced blood homocysteine from 158 nM/ml to 85 nM/ml; however, seizure activities continued. Additional treatment with 100 micro g B12 5X daily and Betaine 200 mg 5X daily resulted in a reduction of plasma homocysteine to 33 nM/ml and CSF 5-methyltetrahydrofolate to 28 nM/L. There was cessation of seizures and normal EEG. The infant showed remarkable developmental progress. It can be concluded that the moderate deficiency of methylenetetrahydrofolate reductase deficiency is not the cause of our patient's illness. However, it cannot be excluded that excessive 5-methyltetrahydrofolate may be the cause of seizures in our patient.

MOLECULAR CHARACTERIZATION OF INBORN ERRORS OF METABOLISM. *A.I. Al-Aqeel.* Dept Pediatrics, Riyadh Military Hosp, Riyadh, Saudi Arabia.

Inherited genetic disorders are quite common in our Saudi community because of the high degree of consanguineous marriages. Biopterin dependent phenylketonuria is a special form of PKU which is quite common in Saudi Arabia, we studied 25 patients with this disorder and we found various mutations leading to variable phenotypes. Vitamin D dependent Ricket's type II is another unique disorder. The c-DNA's from two families were sequenced, it was found that each cell line contained a nucleotide substitution resulting in a stop codon, causing truncated receptor protein of 148 and 291 amino acids which accounts for the severe resistant phenotype. Wilson's disease is another common disease, DNA haplotypes of dinucleotide repeat polymorphism (CA) repeat in the Wilson disease gene have helped us in confirming the diagnosis and in finding heterozygote carriers in one family. We found a unique syndrome, with unique dysmorphic features, bone and joint abnormalities. We mapped the gene for this disorder to 16q11.2-21. In conclusion our Saudi community is quite unique. Molecular genetic studies of these diseases have helped us in further understanding of these diseases in their clinical management and in finding a unique molecular genetic defect at the DNA and protein (enzyme) level.

Mucopolysaccharidosis I: A comparative study of polymorphic DNA haplotypes in three normal Mexican populations. *M.P Gallegos, G.M. Zuniga, L. Sandoval, S.E. Flores, H. Montoya, H. Rangel, M. Casas, F. Rivas, J. Sánchez.* Medicina Molecular y Genetica, CIBO, IMSS, Guadalajara, Jalisco, Mexico.

Mucopolysaccharidosis type I (MPS I) is an autosomal recessive disorder caused by deficiency of the lysosomal enzyme α -L-iduronidase (IDUA). The genetic heterogeneity at the IDUA locus was studied in 100 mutant allele and 720 normal allele in Mexican population (520 mestizos, 100 Tarahumaras, 100 Huichols). For this study three different restriction fragment length polymorphisms (RFLPs) and one VNTR at the IDUA locus were analyzed to search for the frequency of each RFLP produced by KpnI, Eco47III, and NspI restriction endonucleases, and the VNTR fragment. We detected a total of nine haplotypes in both alleles, mutant and normal, the haplotype 1 (1212) was the most common. However two haplotypes were characteristic of the Huichols group, the haplotype 2112 (15%) and 2111 (15%). Our results delineated the molecular heterogeneity of the MPS I haplotypes, as well as their significant interpopulation variation and many carriers of mutant alleles in the majority of the families with one affected child of MPS I.

CLASSICAL GALACTOSEMIA PRESENTING AS PURPURA FULMINANS IN THE NEWBORN. *J.C GAY¹, M.J DASOUKI², L.J ELSAS³*. 1) PEDIATRICS, VANDERBILT UNIVERSITY, NASHVILLE, TN; 2) SECTION OF MEDICAL GENETICS & MOLECULAR MEDICINE.CHILDREN'S MERCY HOSPITALS & CLINICS. KANSAS CITY. MO; 3) DEPARTMENT OF PEDIATRICS. EMORY UNIVERSITY. ATLANTA. GA.

Newborn screening for galactosemia has led to the early diagnosis of most patients and the prevention of fatal hepatotoxicity. However, in some patients the clinical presentation is unusual and the disease progresses before the results of screening are known. In this family of three, the first child survived but was not diagnosed until 16 days of age. He was discharged at age 2 days and readmitted at 6 days of age with purpura fulminans of the right leg and mild disseminated intravascular coagulation. Factor V Leiden, Protein C and Protein S levels were normal and there was no E.coli sepsis. While the first galactosemia screening test was unsatisfactory, the second had a galactose of >50mg/dl and no galactose-1-phosphate uridylyltransferase (GALT) activity. He was diagnosed as G/G galactosemia, placed on no lactose, and recovered rapidly. In a subsequent twin pregnancy, his mother excluded lactose from her diet and underwent early, second trimester amniocentesis. Cultured amniocytes from both amniotic sacs had no GALT activity and DNA was homozygous for the Q188R alleles. At birth, both twins had elevated serum galactose levels [21 and 41 mg/dl] in erythrocytes despite absence of galactose during gestation and before nursing. We conclude that classical galactosemia can present with the unusual clinical disease, purpura fulminans, and that erythrocyte galactose-1-phosphate can be elevated in newborns with Q188R/Q188R galactosemia without exposure to exogenous galactose.

Long Chain 3-Hydroxy Acyl-CoA Dehydrogenase (LCHAD) Deficiency: novel phenotype and genotype abnormalities. *M.J. Dasouki*¹, *C.R. ROE*², *J.A. IBDAH*³. 1) Sect Medical Gen & Molec Med, Children's Mercy Hosp, Kansas City, MO; 2) Institute Of Metabolic Disease. Baylor University Medical Center; 3) Wake Forest University School of Medicine.

The mitochondrial trifunctional protein [MTP] is a hetero-octamer (4 alpha,4 beta subunits) with dehydrogenase, hydratase and thiolase activities that catalyze the second, third and fourth steps of intramitochondrial long fatty acid - oxidation. LCHAD deficiency is a serious pediatric fatty acid oxidation defect with reported maternal and perinatal complications, specifically, maternal AFLP and HELLP syndromes. Here, we report two unrelated families with isolated LCHAD deficiency and distinct phenotype. In family 1, a previously healthy 3-month old girl presented with hepatomegaly, mild hypoglycemia and cardiomyopathy. Despite supportive therapy and early carnitine supplementation, she died within 36 hours of presentation due to irreversible cardiac disease. At 31 weeks gestation, her mother had severe HELLP syndrome and atrial fibrillation. Those symptoms resolved soon after delivery. Our mutation analysis revealed homozygosity for the prevalent G1528C mutation in the MTP alpha-subunit gene. In a following uncomplicated pregnancy, a healthy heterozygous boy was born. In family 2, an 18-month old girl was found to be a compound heterozygote [G1528C; del 261T] after she presented with a hypoglycemic seizure mild hepatomegaly and disseminated intravascular coagulation. There were no reported prenatal complications. In conclusion, while the fetal long chain fatty acid metabolites are thought to be responsible for the associated maternal hepatotoxicity in the AFLP and HELLP syndromes, this study suggests variability of the maternal phenotype. Furthermore, it suggests that fetal metabolites could also be toxic to the maternal heart as seen in family 1.

Subcellular localization of galactose-1-P uridylyltransferase in the yeast *Saccharomyces cerevisiae*. N.C.

*Christacos*¹, *M. Marson*², *L. Wells*³, *K. Riehman*², *J.L. Fridovich-Keil*². 1) Grad Prog Genetics & Mol Bio; 2) Department of Genetics, Emory University School of Medicine, Atlanta, GA 30322; 3) Graduate Program in Biochem. and Mol Bio.

The enzyme galactose-1-phosphate uridylyltransferase (GALT) catalyzes the second step of the Leloir pathway of galactose metabolism, following galactokinase (GALK) and preceding UDP-galactose-4-epimerase (GALE). The potentially lethal disorder classic galactosemia is caused by impairment of GALT in humans. Previous studies have shown that all three Leloir enzymes are released as active, soluble proteins following standard lysis protocols of bacteria, yeast, or mammalian cells. This has led to the historical assumption that all three Leloir enzymes function as free cytosolic enzymes. To test this assumption, we have explored the *in vivo* subcellular localization of GALT in the yeast, *Saccharomyces cerevisiae*. Using Gal7p, the endogenous yeast GALT, covalently tagged with green fluorescent protein (GFP), we found clear evidence of localization of the fusion protein to discrete spots in the cytoplasm of the majority of cells expressing all three Leloir enzymes. In contrast, yeast expressing GFP-Gal7p but lacking either Gal1p(GALK), Gal10p (GALE), or both, did not demonstrate spots in the majority of cells thus, implicating a role, either direct or indirect, for these other Leloir proteins in the localization of Gal7p. GFP-tagged human GALT expressed in yeast also localized to discrete spots, demonstrating that some of the intrinsic determinants of localization have been conserved. These observations raise the intriguing possibility that GALT may function in a sequestered rather than a freely diffusible state, and that this subcellular organization may have been conserved through evolution. Future studies include investigating further the intrinsic and extrinsic factors involved in the localization of Gal7p, and exploring the possible effects of known patient mutations on the localization of human GALT in yeast.

Phenotype characterization of Maleylacetoacetate isomerase deficiency in mouse. *J. Fernandez-Canon, T. Burlingame, M. Batchelor, J. de Koning, M. Al-Dhalimy, M. Gibbison, M. Grompe.* Dept Molecular & Medical Gen, Oregon Health Sci Univ, Portland, OR.

In mammals, the catabolic pathway of phenylalanine and tyrosine is found almost exclusively in liver (hepatocytes) and kidney (proximal tubular cells). There are human diseases associated with deficiencies of all enzymes in this pathway except for maleylacetoacetate isomerase (MAAI). The most severe of these disorders is hereditary tyrosinemia type I (HT1), resulting from deficiency of fumarylacetoacetate hydrolase (FAH) which results in the accumulation of fumarylacetoacetate (FAA) which is converted by an unknown pathway to succinylacetoacetate (SAA) and succinylacetone (SA) which is the diagnostic compound for HT1. Deficiency of MAAI was predicted to result in the accumulation of MAA (cis-isomer) and this compound is thought to be toxic in the same way as its trans-isomer, FAA. It currently remains unclear which human phenotype (disease), if any, is associated with MAAI deficiency. We have generated a mouse knocked out for MAAI activity and we are currently testing the phenotype. Deficient mice accumulate FAA and SA in the urine but, until now, they are healthy and breed normally. They also are sensitive to overload the phenylalanine/tyrosine catabolic pathway. They have oxidative stress (NMO-1 gene induction) which suggest that these mice could be predisposed to cancer when they get older.

The incidence of Smith-Lemli-Opitz Syndrome (SLOS) in Ontario, Canada. *M.J.M Nowaczyk^{1,2}, V.M. Siu³, A.G.W. Hunter⁴, S. Farrell⁵, D. McCaughey², D.T. Whelan^{1,2}.* 1) Departments of Pathology & Molecular Medicine and of Pediatrics, McMaster University, Hamilton, Canada; 2) Hamilton Regional Laboratory Medicine Program, Hamilton, Canada; 3) Children's Hospital of Western Ontario, London, Canada; 4) Children's Hospital of Eastern Ontario, Ottawa, Canada; 5) The Credit Valley Hospital, Mississauga, Canada.

The incidence of SLOS (MIM 279400) has been estimated to be approximately 1 in 60,000 to 1 in 20,000 births in North America. A recent report from Slovakia, where the incidence has been estimated to be 1 in 10,000, found that it was 1 in 20,000 in a fully ascertained population of newborns. Ontario is the most populous province in Canada (11,513,800 inhabitants in 1999; Statistics Canada data). The population is predominantly Caucasian of North European ancestry, with large and varied ethnic groups in the main urban centers. Between June 1, 1999 and May 31, 2000 we have diagnosed five unrelated cases of SLOS: three newborns with clinical features of severe neonatal SLOS, one fetus with severe form of SLOS with cleft lip and palate and holoprosencephaly, and one affected fetus in a family with a previous history of SLOS. In all cases the diagnosis of SLOS was confirmed by demonstration of elevated 7-dehydrocholesterol in plasma (newborns) and in amniotic fluid (fetuses). The two fetuses would have been delivered between June 1999 and May 2000. The ethnic backgrounds were: French-Canadian in two cases, Dutch-Irish, Scottish-Irish, and Croatian. The birth rate for Ontario in the calendar year 1999 was 132,000 (Statistics Canada data). We thus estimate that the minimal incidence of SLOS in Ontario was 1 in 26,500. This number is very likely an underestimate because infants with milder forms of SLOS born during this period remain undiagnosed. The incidence of SLOS in Ontario will be determined further with the introduction of national surveillance for SLOS (Canadian Paediatric Surveillance Program), which started on January 1, 2000. It is hoped that with the increased awareness of the clinical spectrum of SLOS, milder cases will be reported and will allow a more accurate determination of the incidence of SLOS in Ontario.

A heterozygote phenotype is present in the jvs +/- mutant mouse livers. *K. Lahjouji, I. Elimrani, J. Wu, G.A. Mitchell, I.A. Qureshi.* Medical Genetics, Hopital Sainte Justine, Montreal, QC, Canada.

The Juvenile Visceral Steatosis (jvs) mouse, having a mutation in the gene encoding the carnitine transporter OCTN2, is an animal model of autosomal recessive Primary Systemic Carnitine Deficiency (SCD). Like human SCD, the homozygous jvs -/- mice have hepatic and cardiac steatosis, reduced plasma and tissue carnitine levels and increased urinary carnitine clearance. Using jvs mice from Drs Saheki and Hayakawa (Kanazawa University, Japan), we developed a rapid genotyping method using PCR and allele-specific oligonucleotides. Jvs +/- livers had a fatty appearance. Using adult liver samples, we measured the free and esterified carnitine, total cholesterol and triglycerides by radiochemical (Hoppel CL, Techniques in Diagnostic Human Biochemical Genetics, 1991) and spectrophotometric methodology (Zhu Y et al., PNAS, 97:1137, 2000). Our results indicate that there is a significant difference (mean+/- SEM, p less than 0.01) between nonfasting adult normal (n=6) vs heterozygote jvs +/- mice (n=10): free carnitine, 2.28 +/- 0.36 nmol/mg protein vs 0.41 +/- 0.13; total carnitine, 3.48 +/- 0.36 nmol/mg protein vs 1.27 +/- 0.25; triglycerides, 0.14 +/- 0.04 nmol/mg protein vs 0.39 +/- 0.02 and total cholesterol, 0.21 +/- 0.02 nmol/mg protein vs 0.39 +/- 0.04 but not for esterified carnitine, 1.18 +/- 0.17 nmol/mg protein vs 0.9 +/- 0.17. There is also a negative correlation between hepatic free carnitine and triglycerides in liver samples from jvs heterozygotes (P less than 0.05). Our experimental results agree with Scaglia et al., (Genet Med., 1:34, 2000) which show a defective urinary carnitine transport in heterozygote SCD patients and those of Zhu et al., (PNAS, 97:1137, 2000) showing hypertriglyceridemia in mice with heterozygous chromosomal deletion including the OCTN2 region. By extension, we speculate that under severe lipolytic conditions, some SCD heterozygotes might develop clinical symptoms of carnitine deficiency. Supported by MRC Canada.

Detection by newborn screening of asymptomatic, putative short-chain acylCoA dehydrogenase (SCAD)

deficiency. *D.D. Koeberl¹, S. Young¹, M. McDonald¹, G. Vockley², N. Gregersen³, A. Boney¹, D.S. Millington¹.* 1) Div. of Medical Genetics, Dept. of Pediatrics, Duke Univ Medical Ctr, Durham, NC; 2) Dept. Medical Genetics, Mayo Clinic, Rochester, MN; 3) Research Unit Molec. Med., Aarhus Univ. Hosp., 8200 Aarhus N, Denmark.

Tandem mass spectrometry (TMS) was adopted for newborn screening by North Carolina in April 1999, and we have subsequently diagnosed two asymptomatic infants with putative SCAD deficiency. Newborn screening TMS revealed markedly elevated butyrylcarnitine for the initial and repeat bloodspot samples from these infants. Subsequent evaluation included a plasma acylcarnitine profile that revealed markedly elevated butyrylcarnitine, and urine organic acid analysis revealed markedly elevated ethylmalonic acid for both infants. Treatment with carbohydrate, riboflavin and carnitine supplementation was initiated by two months of age. Both infants remain asymptomatic at 6 and 8 months of age; however, butyrylcarnitine is persistently elevated at 4.5 and 7.7 nmol/ml, respectively (normal < 0.48).

By contrast, another infant diagnosed with SCAD deficiency during an evaluation for seizures has residual neurologic deficits. Results for biochemical testing in this patient were very similar to those of the infants mentioned above, but this patient was not born in North Carolina and newborn screening by TMS was not performed. Muscle SCAD deficiency was confirmed in this patient. All three patients have deficient SCAD activity by quantitative acylcarnitine profiling of cultured fibroblasts with [U-¹³C] palmitic acid (Ventura, F. V. et al., Clin. Chim. Acta 281:1-17, 1999). Sequence analysis of the SCAD gene and confirmation of causative mutations in SCAD is pending. We speculate that the early treatment of the first two infants has prevented the expected metabolic crises and neurologic abnormalities associated with SCAD deficiency in previously reported patients. The normal development of two infants with putative SCAD deficiency demonstrates the usefulness of TMS for detection of rare disorders by newborn screening.

Marfanoid habitus with craniosynostosis: Biochemical defect in the conversion of profibrillin-1 to fibrillin-1.

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Marfanoid habitus with craniosynostosis (MFS-C) can be divided into type I, Shprintzen-Goldberg syndrome, and type II (OMIM #182212). We have identified 4 patients with MFS-C type II with Marfanoid body habitus, aortic root dilatation, craniosynostosis, bifid uvula, dolichocephaly, and multiple hernias. All affected individuals were sporadic cases and male. These patients lack the developmental delay, facial features and neonatal hypotonia that characterize Shprintzen-Goldberg patients. Dermal fibroblasts were explanted from MFS-C patients and metabolic labeling studies done to determine if an abnormality could be identified in the cellular processing of fibrillin (fib), the defective protein in Marfan syndrome. Fib is made as a proprotein, profib, and proteolytically processed in the C-terminal domain upon or shortly after secretion. All 4 cell strains demonstrate normal amounts of profibrillin (profib) synthesized and efficient secretion of the protein. All 4 cell strains demonstrated inefficient processing of profib to fib upon secretion. Two hours after metabolic labeling, the majority of total fib in the media was unprocessed profib in the MFS-C cell strains, whereas control cells (n=15) had more processed fib than profib. Twenty hours after metabolic labeling the majority of profib had been converted to fib in the MFS-C cells. Explanted cells from the unaffected parents of 1 patient did not demonstrate inefficient processing of profib, indicating that the biochemical defect was associated with the phenotype. Cells from 2 Shprintzen-Goldberg patients and 2 males with X-linked Marfanoid habitus with mental retardation did not show the biochemical abnormality. These results suggest that MFS-C type II can be characterized by a biochemical defect in proteolytic processing of profibrillin-1 to fibrillin-1.

Compensated Adrenal Insufficiency in Smith-Lemli-Opitz Syndrome. *N.A. Nwokoro¹, K.I. Rother², D.*

Papanicolaou³, G. Chrousos⁴, F.D. Porter¹. 1) HDB/NICHD/NIH, Bethesda, MD; 2) NIDDK/NIH, Bethesda, MD; 3) DEB/NICHD/NIH, Bethesda, MD; 4) PREB/NICHD/NIH, Bethesda, MD.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive MCA/MR syndrome. The Caucasian birth prevalence is between 1/15,000 and 1/60,000. SLOS is associated with a variable but characteristic spectrum of anomalies that include microcephaly, characteristic facies, cleft palate, congenital heart defects, gastrointestinal and renal anomalies, genital malformations (ambiguous genitalia, hypospadias, cryptorchidism), and various limb anomalies. Additionally, mental retardation and a behavioral phenotype with autistic features are seen. SLOS is due to a defect in the conversion of 7-dehydrocholesterol (7DHC) to cholesterol as a result of mutations in the 3 β -hydroxysterol-D⁷-reductase gene. There is accumulation of 7DHC and 8-dehydrocholesterol in tissues and plasma and, in general, reduced tissue and plasma cholesterol levels. Endocrine abnormalities such as sex reversal, hypogonadism, pubertal delay, precocious puberty and, rarely, adrenal insufficiency have previously been noted. We report on the adrenal function of 29 patients (mean age 6 years, range 0.2 to 23 years) with biochemically confirmed SLOS. All 29 patients had normal cortisol responses (>18 mg/dl) to administration of ACTH. Ovine corticotropin releasing hormone tests were performed on 13 of the patients, and 8 patients (62%) had an exaggerated ACTH response. This finding is similar to that seen in patients with hereditary isolated glucocorticoid deficiency who also have compensated adrenal insufficiency. Our results indicate that although none of the SLOS patients had overt adrenal insufficiency, there may be an increased risk of adrenal decompensation in times of severe stress. We speculate that the anomalous cholesterol metabolism in SLOS, which most likely results in abnormal cell membrane composition, interferes with the normal function of the G-protein coupled ACTH receptor. Alternative explanations are decreased bioactivity of ACTH due to disturbed protein processing or altered receptor regulation due to the presence of abnormal sterols produced from 7DHC.

CARNITINE ACYL CARNITINE TRANSLOCASE DEFICIENCY; IS A TREATABLE DISEASE. *R. wanders*¹, *M. Rashid*², *A. Al Aqeel*³. 1) genetics metabolic laboratory, university of amsterdam, amsterdam, netherlands; 2) departemen of biological& medical research, King Faisal Specialist Hospital&Research centre, Riyadh, Saudi Arabia; 3) Departemen of pediatrics, Riyadh Armed Forces Hospital, Riyadh, Saudi Arabia.

We reported a five months old patient who presented on the second day of life with nystagmus and hyperammonemia. Her acyl carnitine profile showed highly elevated C16 and C18 species with normal free carnitine. Her mitochondrial carnitine-acyl carnitine translocase was only 0.2 with the normal (24.7; 35.5 mmol/min.mg). Mutational analysis of this patient revealed 713A-C transversion (Q238R) which is conserved in carnitine acylcarnitine translocase in other species. Expression studies in yeast has to be done to investigate the consequences of this mutation for the function of the enzyme.

Recurrent acute renal failure due to a novel Hypoxanthine Phosphoribosyl Transferase (HPRT) gene mutation.

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A 6-year old boy with partial hypoxanthine phosphoribosyl transferase (HPRT) enzyme deficiency and without the phenotypic features of Lesch-Nyhan syndrome, presented with recurrent acute renal failure from hyperuricemia. Acute renal failure is an unusual presentation for children with HPRT deficiency. Sequence analysis of the HPRT gene cDNA revealed a novel single nucleotide substitution at codon 65 in exon 3 (65CTC>TTC, leu>phe). This mutation was also detected in his asymptomatic mother and sister. Unlike the cells from patients with classic Lesch-Nyhan syndrome, the in vitro cultures of the patients T-lymphocytes did proliferate in the presence of the purine analogue 6-thioguanine. The ability to grow the lymphocytes in vitro allowed the cDNA sequencing which revealed the HPRT mutation. This report highlights the occurrence of recurrent acute renal failure in a child with partial HPRT enzyme deficiency, in which the newly described mutation in HPRT did not confer resistance to 6-thioguanine. The absence of 6-thioguanine cytotoxicity shows that even small alterations in the enzyme activity in vivo can result in disease symptoms. Atypical HPRT mutations should also be considered in cases of unusual renal failure, because correct diagnosis can allow appropriate treatment, as well as informed genetic counseling.

Characterization of molecular defects in Korean families with inherited ornithine transcarbamylase deficiency and their genotype-phenotype correlations. *H.W. Yoo¹, G.H. Kim¹, J.W. Cheong², E.J. Seo¹.* 1) Medical Genetics Clinic & Laboratory, Asan Medical Center, Ulsan University College of Medicine, Seoul, Korea; 2) Asan Institute for Life Sciences, Asan Medical Center, Seoul, Korea.

Ornithine transcarbamylase(OTC) deficiency is one of the most common inborn error of urea cycle, which is inherited in x-linked manner. OTC deficiency leads to the accumulation of ammonia, hyperammonemia resulting in fatal damage to central nervous system. This study was undertaken to characterize molecular defects in 20 unrelated Korean families with OTC deficiency, correlate genotypes with phenotypes based on clinical features and results of in vitro expression study. To investigate molecular lesions resulting in OTC deficiency, the OTC genes of probands were amplified exon by exon and analyzed by direct sequencing of double stranded DNA. To analyze expression in vitro, mutant OTC cDNAs were constructed and cotransfected with b-galactosidase gene into COS-1 cells by using lipofection. After transient expression, OTC activity was assayed by colorimetric method. We identified 14 different mutations in 20 unrelated Korean families with OTC deficiency; R26X, T44I, R92X, G100R, R141Q, G195R, M205T, H214Y, D249G, R277W, F281S, R320X, C deletion at nt 853, and 10 base pair deletion at nt 796-805, involving well conserved nucleotide sequences across species or CpG hot spots. Among these mutations, all the mutations except G100R and R277W were identified in patients with neonatal onset expressing no residual enzyme activities in vitro. The R277W mutation has been well known to be associated with late onset OTC deficiency. However, the G100R mutation is a novel mutation causing late onset OTC deficiency. The genotypes of Korean patients with OTC deficiency are genetically private as in other x-linked inherited disorders.

³¹P-nuclear magnetic resonance spectroscopy (NMRS) in hypophosphatasia: Diagnostic urine profile indicating multiple new substrates for bone alkaline phosphatase. *M.P. Whyte^{1,2}, M.C. Eddy¹, M.N. Podgornik¹, A.*

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Hypophosphatasia (HPP) features low serum alkaline phosphatase (ALP) activity and impaired skeletal mineralization (rickets or osteomalacia) due to deactivating mutations in the gene that encodes the tissue non-specific ALP isoenzyme (TNSALP). To date, 3 phosphocompounds are known to accumulate endogenously in HPP patients: phosphoethanolamine (PEA) and inorganic pyrophosphate (PPi) in urine, and pyridoxal 5'-phosphate (PLP) in plasma. PEA, PPi, and PLP seem, therefore, to be natural substrates for TNSALP. Excess PPi, a mineralization inhibitor, may account for the skeletal disease of HPP. Nevertheless, the physiological role of TNSALP is incompletely understood.

To search for additional natural substrates for TNSALP, we performed ³¹P-NMRS using urine from 11 children with HPP and contrasted the spectra to 2 healthy controls and 8 patients representing 5 other rachitic disorders. Urine from controls and non-HPP rickets showed the anticipated, single, major ³¹P-resonance peak consistent with inorganic phosphate (Pi) and only low levels of phosphocompounds. In contrast, HPP urine invariably contained 5-6 additional peaks in 10-50 fold excess. Spiking experiments tentatively show a peak for PEA, PPi, and phosphocholine. Ongoing NMR experiments will clarify and confirm their identity.

³¹P-NMRS reveals elevated levels of multiple phosphocompounds in the urine of HPP patients diagnostic for this inborn error of metabolism. Importantly, ³¹P-NMRS of HPP patients indicates that there are several previously unrecognized (new) natural substrates for TNSALP. This discovery should enhance understanding of the physiological role of TNSALP and provide a means to assess potential medical treatments for HPP.

Phenotypic, Biochemical, and Neurophysiological Characterization of a SLOS Mouse Model. *F.D. Porter¹, P. Zhu², L. Kratz³, P.A. Krakowiak¹, K. Battaile⁴, R.D. Steiner⁴, R.R. Stewart², R.I. Kelley³, N.A. Nwokoro¹, C.A. Wassif¹.*
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Smith-Lemli-Opitz syndrome (SLOS) is a MCA/MR syndrome due to mutations in the 3 β -hydroxysterol D⁷-reductase gene (*DHCR7*) which leads to impaired conversion of 7-dehydrocholesterol (7DHC) to cholesterol. A mouse model of SLOS was produced by disruption of *Dhcr7* in ES cells. *Dhcr7*^{-/-} pups, similar to infants with SLOS, demonstrated growth failure (77% control), dysmorphic features, decreased movement, and feeding problems. Dysmorphology included cleft palate (9%) and nasal plug retention (39%). Mutant pups did not spontaneously feed. Hand feeding showed an uncoordinated suck and aspiration. 3 β -hydroxysterol D⁷-reductase activity in mutant fibroblasts, measured by reduction of ergosterol, was 0.3% of control levels. Tissue sterol profiles showed decreased cholesterol and increased 7DHC levels in *Dhcr7*^{-/-} pups. In brains from *Dhcr7*^{-/-} embryos we found that 7DHC levels were similar to control cholesterol levels, and that there was a progressive increase of 7-dehydrodesmosterol (7DHD) which paralleled the normal developmental increase of desmosterol. Neurophysiological testing showed that neurons from the frontal cortex of *Dhcr7*^{-/-} pups exhibited a normal sodium current, were able to generate an action potential in response to a depolarizing current, and responded to GABA. In contrast *Dhcr7*^{-/-} cortical neurons showed minimal (-13 ± 1 pA, n=5) or no response (n=5) to glutamate stimulation. The control glutamate response was -46 ± 9 pA (n=8). We postulate that this impaired glutamate response was due to receptor dysfunction as a consequence of the substitution of 7DHC and 7DHD for cholesterol in the plasma membrane. Neurological dysfunction including hypotonia, mental retardation, and behavioral problems are major findings in SLOS. Perturbation of neurotransmitter function may underlie these problems. This mouse model will be useful in determining the biochemical and neurophysiological basis of the behavioral and learning problems seen in this MCA/MR syndrome as well as testing therapeutic interventions.

Is a new very long-chain acyl-CoA synthetase, the "bubblegum" protein, involved in X-linked adrenoleukodystrophy? *P.A. Watkins, S.J. Steinberg, J. Morgenthaler, N. Oey, A.K. Heinzer, K.D. Smith.* Kennedy Krieger Institute and Johns Hopkins University Sch. Med., Baltimore, MD.

Neurodegeneration and elevated tissue levels of saturated very long-chain fatty acids (VLCFA), phenotypic features of X-linked adrenoleukodystrophy (XALD), were observed in the "bubblegum" mutant of *D. melanogaster* (KT Min and S Benzer, Science 284, 1985-8, 1999). To determine whether the product of the *bubblegum* gene was involved in XALD, we cloned full-length cDNA for the human gene. It encoded a 724 amino acid protein (hBG) containing an AMP-binding domain characteristic of acyl-CoA synthetases. Following expression of cDNA in COS-1 cells, hBG catalyzed the activation of VLCFA and long-chain fatty acids (LCFA) to their CoA derivatives. Several monounsaturated and polyunsaturated fatty acids, including docosahexaenoic acid which is necessary for normal brain development, were also found to be hBG substrates. Interestingly, hBG belongs to a different protein family than does human very long-chain acyl-CoA synthetase (hVLCs), the only other enzyme with significant capacity to activate VLCFA. By Northern analysis, a 3.0 kb hBG mRNA transcript was detected primarily in brain. A less abundant but larger (6.4 kb) transcript was detected in muscle, heart and skin fibroblasts. Overexpression of hBG in fibroblasts from XALD patients had no significant effect on VLCFA metabolism. However, Northern blots revealed decreased *bubblegum* gene expression in XALD fibroblasts. A similar result was obtained using by reverse transcription-PCR. Further studies to elucidate the role, if any, of hBG in the biochemical pathology of XALD are in progress. (Supported by NIH grants HD10981, NS10533 and NS37355).

Peroxisomal mosaicism as a diagnostic dilemma. *E.M. Garcia-Soto^{1,2}, G.V. Raymond^{1,2}, H. Gilmore³, A.B. Moser^{1,2}, P. Watkins^{1,2}, S. Mihalik^{1,2}.* 1) Dept Neurogenetics, Kennedy Krieger Inst, Baltimore, MD; 2) Johns Hopkins University, School of Medicine, Baltimore, MD; 3) Baystate Med Ctr Child Hsp, Springfield, MA.

Diagnosis of mild the forms of peroxisomal biogenesis disorders (PBD) can be very difficult. Even though patients with frank Zellweger syndrome are readily identifiable, those with the milder phenotypic variants such as neonatal adrenoleukodystrophy (NALD) and infantile Refsum disease (IRD) cannot be easily differentiated either biochemically or phenotypically at the early stages of the disease. Their diagnostic parameters are often just outside the normal range and their phenotype is particularly difficult to differentiate from those patients with single enzyme defects (SED) of peroxisomal fatty acid oxidation. With complementation analysis, 12 PBD groups have been identified and all variants of PBDs have been found within a single complementation group. We describe a patient who presented clinically at age of 9 months with hypotonia and a history of feeding difficulties, hearing problems and blindness since birth. Initially analyses of metabolites in plasma suggested a SED: increased cerotic acid (C26:0), decreased essential fatty acids such as arachidonic and docosahexaenoic acid, and normal phytanic acid levels. Plasmalogen synthesis was borderline normal in his first fibroblast biopsy. In further analyses, which included fibroblasts from two different biopsies, we found different peroxisomal populations. For example, catalase was not soluble from fibroblasts in the first biopsy but soluble in the second set of fibroblasts. There was both normal and abnormal processing and expression of peroxisomal β -oxidation proteins and plasmalogen biosynthesis was clearly abnormal in the second biopsy. The patient was assigned to group-1 by complementation analysis. It is important to consider that peroxisomal mosaicism may lead to variability in peroxisomal diagnostic parameters, making the final diagnosis difficult in patients presenting with mild phenotypes at the early stages of disease. (Supported by NIH Grant HD10981).

Molecular characterization of patients with chondrodysplasia punctata. *N. Brunetti-Pierri¹, G. Meroni², M.R. Tuzzi¹, M.V. Andreucci¹, G.R. Vega¹, A. Ballabio², G. Andria¹, G. Parenti¹.* 1) Dept. of Pediatrics, Federico II University, Naples, Italy; 2) Telethon Institute of Genetics and Medicine, Milan, Italy.

Skeletal disorders associated with chondrodysplasia punctata (CDP) are characterized by clinical, genetic and biochemical heterogeneity. Defects of arylsulfatase E; delta7,delta8 hydroxysteroid isomerase; and 3-beta-hydroxysteroid dehydrogenase have been detected in patients with X-linked recessive CDP (CDPX1), X-linked dominant CDP (Conradi-Hunermann syndrome or CDPX2) and CHILD syndrome, respectively. Defects of peroxisomal function are responsible for the autosomal recessive rhizomelic CDP. In a series of 26 patients with CDP we have performed SSCP analysis and sequencing of the arylsulfatase E gene (26 cases), of the delta7,delta8 hydroxysteroid isomerase gene (17 cases) and of the arylsulfatase F gene (also localized in the critical region for CDPX1)(20 cases). We have detected 4 mutations of the arylsulfatase E gene: a missense mutation (I80N), an insertion (Tins616), two nonsense mutations (R540X, W581X). Three of these patients presented with phenotypes compatible with CDPX1 (nasal and mid face hypoplasia and brachytelephalangy). One of them also had sensorineural deafness. The fourth case was an aborted fetus presenting with severe chondrodysplasia punctata and brachytelephalangy. The CDPX1 phenotype, commonly considered relatively mild, may possibly include more severe clinical manifestations, as those observed in CDPX2. Sensorineural deafness might also be considered part of the CDPX1 phenotype. No mutations of the delta7,delta8 hydroxysteroid isomerase and of the arylsulfatase F genes were detected. Our negative results in most of the subjects studied support the idea that other so far unknown metabolic defects might be responsible for CDP. This work was supported by the Italian Telethon Foundation (grant E.790 to G.P.) and by MURST, Rome (PRIN 1998 to G.A.).

Enhanced Chondrocyte Apoptosis Leads to Abnormal Matrix in the Cartilage of MPS Rats and Cats. C.M.

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A major feature of the mucopolysaccharidoses (MPS) is abnormal cartilage and bone development leading to short stature, dysostosis multiplex, and degenerative joint disease. Ultrastructurally, the cytoplasm of affected MPS chondrocytes is filled with membrane bound vacuoles containing undegraded glycosaminoglycans (GAGS). Clusters of hypertrophic chondrocytes form in these disorders associated with disruption of the normal cartilage organization. To further investigate the cellular pathology of the MPS disorders, primary cultured chondrocytes and cartilage were examined from cats and rats with MPS Type VI (Maroteaux-Lamy disease), and cats with MPS Type I (Hurler disease). Increased numbers of apoptotic chondrocytes were identified by TUNEL staining and/or the presence of empty lacunae in the articular cartilage and growth plates of the MPS animals as compared to age-matched controls. Marked proteoglycan depletion also was observed in the MPS epiphyses by Safranin-O staining, and the presence of excess collagen type II was detected by immunostaining. A model of MPS endochondryl bone growth pathology is proposed in which lysosomal dysfunction (due to primary lysosomal enzyme deficiencies) leads to an enhanced propensity for chondrocyte cell death. In turn, the MPS cartilage matrix around these dead and dying chondrocytes contains increased amounts of undegraded collagen type II and a depletion of proteoglycans (particularly in the epiphyses). Novel treatment strategies for these disorders might therefore be considered to prevent chondrocyte apoptosis as an alternative to replacement of the missing enzymatic activity.

A novel mtDNA mutation (T9176G) hampers ATP production and results in Leigh syndrome. *R. Carrozzo, A. Tessa, F. Piemonte, G. Chichierchia, C. Patrono, O. Capuano, C. Dionisi-Vici, E. Bertini, F.M. Santorelli.* Molecular Medicine Unit, Bambino Gesù Hosp, Rome, Italy.

Leighs syndrome (LS) is a severe neurodegenerative disorder of infancy or childhood. associated with different defects of energy metabolism affecting ATP production. In addition to defects in COX, PDH, and complex I, mutations in the mitochondrial DNA (mtDNA) are an important etiology of LS. The extent to which mtDNA alterations affect cellular production of ATP is largely unknown. For instance, cellular characterization has been shown only for the mtDNA T8993G, a common cause of maternally inherited LS. We identified a family in whom a 10-year-old girl developed a severe neurodegenerative disorder resembling LS. Her sister had died of pathologically confirmed LS. Both girls harbored high percentages of a novel ATPase 6 mutation (T9176G). The mutation fulfilled accepted criteria for pathogenicity. The effects of the mutation in tissues and cells were investigated by using different approaches. ATPase activity in digitonized skin fibroblasts from the proposita showed a slightly, though not statistically significant, lower rate than control cell lines. This activity in cell lines was highly oligomycin sensitive. Conversely, ATP synthesis rates were severely hampered. The residual activity was about 20% when we used succinate and negligible using malate as substrate to start the reaction. ATP synthesis was fully inhibited by oligomycin. Immunocytochemical studies disclosed a severely hampered ATP rate in skin fibroblasts cultures from the proposita. The morphology of fibroblast cell lines and the ability of their mitochondria to generate a valid membrane potential was monitored by fluorescence microscopy adding the dye JC-1. More than 10% of the patients cells were consistently seen to have very low membrane potential with a greater proportion having a less severe decrease. Finally, two-dimensional electrophoresis revealed that all OXPHOS complexes were normally assembled in skeletal muscle homogenate from the proposita except complex V as compared to normal controls. Our findings expand the genetic variants associated with LS and suggest clues as for the pathogenesis.

Possible mechanism of nerve damage on hyperphenylalanine in embryonic rat. *X.F. Gu, X.W. Yang, R.G. Chen.*
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OBJECTIVE Children with phenylketonuria are mental retardation. This study is to investigate of neurotoxicity of hyperphenylalanine and to explore of possible mechanisms. **METHODS** The primary cortical and hippocampal neurons of embryonic rat were cultured in serum-free medium, exposed to hyperphenylalanine (Phe) and assayed the survival rate. The morphological changes were observed by special staining. nNOS, bcl-2, fas and c-fos were tested by immunohistochemistry and RT-PCR. **RESULTS** The survival rate of cortical neurons was significantly decreased compared to normal controls in present Phe at 300 $\mu\text{mol/L}$, 600 $\mu\text{mol/L}$, 1200 $\mu\text{mol/L}$ and shown a dose dependent. Positive apoptotic neurons increased with the concentration of Phe by TUNEL staining. The positive neurons of nNOS, fas and c-fos increased than normal control by immunohistochemistry with the exception of bcl-2. The mRNA expression of nNOS, fas, and c-fos increased accordingly while that of bcl-2 decreased. **CONCLUSION** The hyperphenylalanine is neuronal toxin to the central nervous system. The over production of nitric oxide may take an important part in the damage of neurons. The abnormal expression of some genes may hasten the neuronal apoptosis, such as the upregulation of fas and c-fos genes and the downregulation of bcl-2.

Very-long-chain acyl-coenzyme A dehydrogenase(VLCAD)deficiency first presenting with hypoglycemia and strokes. *P. Jayakar¹, D. Martinez², C.R. Roe³, S. Rarback⁴, S. Lazar⁵*. 1) Genetics & Metabolism, Miami Children's Hosp, Miami, FL; 2) Dept of Neurology, Miami Children's hospital, Miami, FL; 3) Dept of Genetics, Baylor College of Medicine, Dallas, TX; 4) Dept of Pediatrics/Nutrition, University of Miami, Miami, FL; 5) Division of Pediatric critical care,JD children's hosp,Hollywood,FL.

VLCAD is an increasingly recognized mitochondrial disorder presenting with episodes of metabolic decompensation. We present a previously healthy 16 mth old male born to Cuban/German non-consanguineous parents who first came to the emergency room with an episode of vomiting and poor oral intake. He was unresponsive, with seizures and hypoglycemia (15mg/dl). He was given D25%, intubated and placed on pentobarbital coma for status epilepticus. Clinical exam revealed a left hemiparesis, hepatomegaly, and no cardiomyopathy. Initial tests showed ketonuria, no acidosis, elevated liver function tests, negative toxicology screen, normal thromboembolic profile and CSF. MRI brain revealed an acute infarction in right frontal-temporal region. Total and free carnitine profiles were low. Neogen screen showed elevation of long chain ratios, low short chain acylcarnitine and low free carnitine. Skin fibroblasts incubated with d3-palmitate and L-carnitine confirmed VLCAD. DNA analysis revealed heterozygosity for A628C which changes threonine 170 to proline(unique mutation) and C1837T in exon 20 which changes arginine 573 to tryptophan, confirming the diagnosis of VLCAD. Fibroblasts for acyl-coA-dehydrogenase of mitochondrial fatty acid oxidation showed 0.19nmols reduced ETF/min/mg prot(control 1.64 +/- 0.57).He was immediately started on IV carnitine, IVF and later switched to Portagen with polycose, MCT oil and fat restricted diet. His clinical improvement was slow, the hepatomegaly resolved but occasional seizures and mild hemiparesis have still persisted. This to our knowledge is the first case of VLCAD presenting with hypoglycemia and strokes, further emphasizing the known phenotypic heterogeneity of this metabolic disorder.Early diagnosis and prompt intervention are critical to avert long term neurologic deficits.

Isolated Sulfite Oxidase Deficiency: Manitoba Experience. *C. Prasad¹, C.R. Greenberg¹, L.A. Dilling¹, W. DeGroot¹, J.B. Carson¹, L.E. Seargeant¹, F.A. Booth¹, K.V. Rajagopalan², J.L. Johnson².* 1) University of Manitoba, Winnipeg, Canada; 2) Dept. Biochemistry, Duke University Medical Centre, Durham, NC, USA.

Isolated sulfite oxidase deficiency is a rare autosomal recessive inborn error of sulfur metabolism. Clinical features generally include devastating neurologic dysfunction, dislocation of eye lenses, and increased urinary excretion of sulfite, thiosulfate, and S-sulfocysteine. There remains a risk of missed diagnosis due to variability in sulfite and thiosulfate test results. We present clinical and molecular data on three patients with isolated sulfite oxidase deficiency. Two patients belong to an aboriginal genetic isolate community in Manitoba. Both patients (1 male and 1 female {deceased}) developed neonatal seizures and progressive neuro-developmental delay. The third patient, (female) born to consanguineous parents of Filipino background, presented with developmental delay at 4 months of age. Her eye lenses are partially dislocated. Based on increased urinary excretion of sulfite, thiosulfate, and S-sulfocysteine, and normal serum uric acid levels, sulfite oxidase deficiency was suspected in all three. The 2 aboriginal patients have a four base pair deletion, 1347-1350delTTGT in the sulfite oxidase gene (SUOX), predicting a premature termination of the sulfite oxidase protein. Termination occurs well into the third domain of the sulfite oxidase protein which contains most of the contact sites essential for enzyme dimerization. The C-terminal portion of this domain, which is absent in these patients, is important in this regard. The third patient is homozygous for a single-base substitution, 1423C®T, which predicts the single amino acid substitution L475F affecting the third domain near the end of the sulfite oxidase protein. This conservative mutation probably disrupts secondary and tertiary protein structure to a lesser extent than the early termination in the aboriginal patients. Therapeutic options are limited to dietary manipulation using formulae low in cystine and methionine and symptomatic treatment for sequelae. We plan to offer carrier testing in the aboriginal genetic isolate using the 1347-1350delTTGT mutation.

Neurologic features of alpha-mannosidosis in the cat. *C.H. Vite¹, M.E. Haskins¹, J.C. McGowan², J.H. Wolfe¹*. 1) Dept Pathobiology, Univ Pennsylvania-Sch Vet Med, Philadelphia, PA; 2) Dept Radiology, Univ Pennsylvania- Sch Med, Philadelphia, PA.

Alpha-mannosidosis is a disease caused by the dysfunction of alpha-mannosidase, a lysosomal hydrolase involved in the degradation of glycoproteins. The disease is characterized by the accumulation of mannose-rich oligosaccharides within lysosomes. The purpose of this study was to determine the neurologic features of alpha-mannosidosis in a colony of cats housed at the University of Pennsylvania School of Veterinary Medicine. Three affected and three unaffected cats from two litters were examined weekly from 4 weeks until death at 18 weeks of age. The first abnormality noted in affected cats was narrowing of the palpebral fissure and a fine whole-body tremor first obvious at 5 weeks of age. Over time, additional signs developed including intention tremor of the head and limbs; coarse, whole body tremors; truncal ataxia; lack of development of a menace response; loss of balance; and nystagmus. Other signs included slow weight gain, flattening of the sternum, flattening of the face, and gingival hyperplasia. Electrodiagnostic testing, electroencephalography (EEG), and magnetic resonance (MR) imaging were used at multiple timepoints to characterize the neurologic dysfunction. Affected cats showed: slow motor nerve conduction velocity (38 m/s; S.D. 1.4 in the tibial nerve) indicative of demyelination; an increase in central conduction time (3.18 ms; S.D. .07) as identified by brain stem auditory evoked response testing; and diffuse slow wave activity identified using EEG. MR imaging of affected cats revealed diffuse white matter signal abnormalities throughout the brain; magnetization transfer contrast, a method used to quantify the amount of water bound to macromolecules in the brain, was decreased by approximately 15% indicating myelin abnormalities within the brain. These data give evidence of defective myelination in both the peripheral and central nervous system in cats affected with alpha-mannosidosis. These findings will serve as control data for future studies involving gene transfer to treat this neurodegenerative disorder. Supported in part by NIH (NS-02032; RR-02512).

Subcellular Localization of the Barth Proteins. *T.J. Phipps¹, D. Hirsch-Shell², L. Hamilton¹, V. Pureza¹, A. Metzenberg¹.* 1) Biology, Cal State Northridge, CA; 2) Massachusetts Institute of Technology, Boston, MA.

Barth Syndrome is characterized by heart failure in the neonatal period, cardiomyopathy, cyclic neutropenia, urine aciduria, abnormally shaped mitochondria, and poor muscle tone. Barth Syndrome usually follows an X-linked recessive pattern of inheritance, and linkage in some families to Xq28 led to the discovery that mutations in the BTHS gene can cause Barth Syndrome. The gene is differentially transcribed from two different start sites, and transcripts are alternatively spliced, such that only products encoded by transcripts with the exon 1 start site are predicted to include a highly hydrophobic domain that may serve as a membrane anchor. The normal functions of the BTHS proteins are not known, but homology searches suggest that the BTHS gene may encode a lipid acyltransferase. The abnormalities in mitochondrial structure led us to hypothesize that the hydrophobic products are anchored to the inner membrane of the mitochondria, whereas the proteins lacking the hydrophobic domain are likely to be distributed more evenly throughout the cytosol. The goal of this study was to use polyclonal antibodies to determine the subcellular localization of the hydrophobic isoforms in wild type cells. We successfully raised two different sets of antibodies, one to the N-terminal region (called the anti-BTHS1-2.2 set) and the other to the C-terminus (called the anti-BTHS10-11 set). The anti-BTHS1-2.2 set should only recognize the membrane-associated protein isoforms, while the anti-BTHS10-11 should recognize all possible isoforms. Using Western analysis and immunocytochemistry, BTHS isoforms were distinguishable in cultured human lymphoblastoid, human fibroblastoma cells, as well as adult human leukocytes. It appears that the hydrophobic isoforms are located in the mitochondria and the plasma membrane of these cells. The explanation for the differential negative effects of absent or abnormal BTHS proteins on the mitochondrial membrane compared to the cytoplasmic membrane may be a threshold effect. We suggest that the search for the function of the BTHS gene products should be focused on the membrane associated forms, especially in the mitochondria.

Functional analysis of the mouse gene for galactose-1-phosphate uridyl transferase (GALT). *N.D. Leslie, S. Bai.*
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Galactose-1-phosphate uridyl transferase (GALT) is expressed in most tissues. However, augmentation of catalytic activity is found during the immediate perinatal period, and changes of steady state mRNA levels are found to change with age and with tissue type. To further characterize the regulation of the mouse GALT gene, we isolated and sequenced over 3 Kb of 5 prime flanking sequence and characterized luciferase reporter expression in cultured cell lines. To further evaluate the promoter region in vivo, we produced mice carrying a mouse GALT: luciferase transgene. The minimal promoter was characterized in vitro and found to contain regions of homology with rat and human GALT promoter regions. Transgenic mice expressed reporter activity with increased activity in the perinatal period. Tissue specificity varied from that of the endogenous GALT message. To determine if galactose loading could induce the GALT promoter, we bred the transgene into both wild type and GALT knockout mice, then exposed both lines to high galactose chow. High tissue galactose levels failed to increase reporter activity in either wild type or knockout mice, and the endogenous GALT message was not increased in wild type mice. These experiments show that GALT regulation is complex and not directly correlated with high substrate levels.

Gene defect in a lethal neonatal metabolic syndrome with iron accumulation: analysis of positional candidate genes. *I. Visapaa*^{1,2}, *V. Fellman*³, *V. Kumar*⁴, *J. Hutton*⁵, *K. Raivio*³, *M. Makarow*⁴, *G. Payne*⁵, *L. Peltonen*^{1,2}. 1) UCLA Dept. of Human Genetics, Los Angeles, USA; 2) Dept. of Human Molecular Genetics, National Public Health Institute and Dept. of Medical Genetics, University of Helsinki, Helsinki, Finland; 3) Hospital for Children and Adolescents, Helsinki, Finland; 4) Institute of Biotechnology, University of Helsinki, Helsinki, Finland; 5) UCLA Dept. of Biological Chemistry, Los Angeles, USA.

We have previously mapped a lethal neonatal syndrome characterized by intrauterine growth retardation, lactic acidosis, aminoaciduria and liver hemosiderosis to chromosome 2q33-37 using genome wide scan in Finnish families. This syndrome is inherited by autosomal recessive mode and it has been so far reported only in Finland. We restricted the disease locus to an 1 cM region using linkage disequilibrium and ancestral haplotype analyses. Since all families shared one ancestral haplotype, most probably every family carries the same founder mutation. Defect in cellular respiratory system and/or iron metabolism has been speculated to cause this disease, but so far the pathogenesis is unknown. Based on radiation hybrid mapping, BCS1 is a promising candidate gene assigned to this critical chromosomal region. The homologous yeast protein, Bcs1p, is a chaperone for the assembly of the cytochrome bc1 complex in the mitochondrial respiratory chain. Another tempting candidate gene locating on the critical chromosomal region based on radiation hybrid mapping is MTABC3, a novel mitochondrial transporter involved in iron homeostasis. We have so far sequenced the human BCS1 gene, and found a single amino acid change, which is present in all disease chromosomes and not found in 260 regionally selected control chromosomes. To find out, if this point variation represents the disease mutation or a polymorphism linked to the disease gene, we are carrying out expression and complementation studies in yeast. Further sequence and functional analyses of these genes will hopefully expose the gene defect resulting in this interesting lethal disease.

Molecular analysis of human carnitine palmitoyltransferase II (CPT II) deficiencies in the *Pichia pastoris* expression system. *N. Chen*¹, *M. Hollingsworth*², *D. Smail*¹, *G.D. Vladutiu*¹. 1) Pediatrics, State Univ. of NY at Buffalo, Buffalo, NY; 2) Biological Sciences, State Univ. of NY at Buffalo, NY.

Carnitine palmitoyltransferase II (CPT II) is one of three enzymes responsible for the transport of long-chain fatty acids into the mitochondrial matrix for beta-oxidation. CPT II deficiency causes the most common lipid myopathy in humans. The adult-onset form of the disease is characterized by muscle pain, stiffness, rhabdomyolysis and myoglobinuria. We have previously reported that heterozygosity for a novel mutation, R503C, in exon 4 of the *CPT2* gene, confers malignant hyperthermia and a variable myopathy. To test the hypothesis that this mutation alters the enzyme's activity causing the observed symptoms, human wild-type and mutant cDNAs were cloned by RT-PCR from human skeletal muscle and expressed in a *Pichia pastoris* protein expression system. A cDNA "cassette system" was established to insert specific mutations into the wild-type *CPT2* gene by restriction substitution using exon 4 from a patient with a known mutation. Exon 4 contains the majority of reported mutations in the *CPT2* gene that can be easily identified by PCR amplification of genomic DNA and allele-specific oligonucleotide analysis. Expressed wild-type or mutant CPT II enzyme activities were assayed using the isotope exchange method. Initial studies have shown that the expressed CPT II enzyme containing the R503C mutation has lower specific activity than the wild type enzyme. This finding supports our hypothesis that the R503C mutation in the human *CPT2* gene causes a reduction of the CPT II enzyme activity and is likely responsible for the myopathic features observed in patients carrying this mutation. The methodology can be expanded to study the impact of other mutations on *CPT2* gene expression (Supported by the Muscular Dystrophy Association and the Children's Guild of Buffalo).

Coding Sequence Mutations in Carnitine Transporter Genes, CACT and OCTN2, exert Long Distance Effects resulting in Aberrant Splicing and Carnitine Deficiency. *B. Hsu¹, S. Koo-McCoy¹, Z. Wang¹, S. Cederbaum², V. Iacobazzi³, F. Palmieri³, CA. Stanley¹, A. Ganguly⁴.* 1) Div. of Endocrinology, CHOP, Philadelphia, PA; 2) MRRC Los Angeles, CA; 3) University of Bari, Bari, Italy; 4) Department of Genetics, University of Pennsylvania, PA.

OCTN2 and CACT transport carnitine and acyl-carnitine esters across the plasma membrane and mitochondrial inner membrane, respectively, for fatty acid beta-oxidation and ketogenesis. Infants with recessive mutations of either of these carnitine transporters present with life-threatening attacks of hypoketotic hypoglycemic coma, chronic cardiomyopathy, and muscle weakness. The molecular basis of the disease was interrogated at genomic and cDNA level in one child with deficiency of the plasma membrane carrier and three with deficiency of the mitochondrial membrane carrier. For OCTN2 and CACT genes, respective exons and exon-intron boundaries were sequenced. Gene specific primers were used to generate cDNA from lymphoblasts or fibroblast RNA. Eight mutations were found at the genomic level. Two individuals were homozygous carriers of two independent frameshift mutations(FS) in OCTN2 and CACT respectively. The remaining two individuals were each compound heterozygotes for a FS mutation and an intronic mutation (T-10->G-10 in intron 2) in CACT gene. All three FS mutations are predicted to translate to premature stop codons. The intron mutation, T-10->G-10, of CACT gene can perturb the branch point location and alter splicing. Analysis of cDNA revealed novel splicing events different from the predictions at the genomic level. All three FS mutation-containing alleles of CACT gene give rise to skipping of exon 3. In contrast, the FS mutation in exon 4 of OCTN2 gene redefines exon 3. It activates the use of two cryptic splice sites in intron 3 and results in inclusion of 13 or 19bp from the 5'-end of intron 3. This translates into an in-frame insertion of 56 unique amino acids between the fourth and fifth transmembrane domains of OCTN2 protein. The results suggest that a coding sequence mutation can lead to either premature protein truncation or a loss of function mutation when aberrant splicing becomes the main effect.

Myopathy in Glycogen Storage Disease Type III. *P.S. Kishnani, A. Kelly, S. Bliss, M. Wojtkiewicz, L. Champion, L. Case, W. Smith, Y.T. Chen.* Duke Univ Medical Ctr, Durham, NC.

Genetic deficiency of Glycogen debranching enzyme causes Glycogen Storage Disease (GSD III). The disease is characterized by hepatomegaly, hypoglycemia, short stature, variable myopathy and cardiomyopathy. Most patients have disease involving both liver and muscle (type IIIa), some (~15 % of all GSD III) have only liver involvement (type IIIb). Patients with GSD III vary remarkably, both clinically and enzymatically. Liver symptoms improve with age. Muscle weakness, though minimal during childhood, may become predominant in adults with onset in third or fourth decade, and there is remarkable clinical variability even within patients with myopathy. 14 individuals (11 GSD IIIa, 3 GSD IIIb, 7F: 7M, age range 16 months-63 years, mean age 17.5yrs) were tested. 8 patients older than 8 years were strength tested for 10 key muscle groups using dynamometry. No difference in strength between the right and left sides was noted in any of the patients studied. Within the adult population tested overall strength was 60% of normal for GSD IIIa and 72% of normal for GSD IIIb. For both groups, hip abduction was the strongest; knee extension and grip were the weakest muscle groups. Knee extension for type IIIa patients was 54% of normal, grip was 53% of normal. For type IIIb patients, knee extension was 45% of normal and grip 61% of normal. Under strenuous exercise 1 GSD III b patient developed a CPK >1500 (norm < 200U/L). Six patients less than age 8 (5 IIIa, 1 IIIb) were evaluated using the Peabody Developmental Motor Scales (PDMS). On the PDMS delayed fine motor skills with increasing age was noted. The two youngest children (16 and 23 months) were age appropriate for fine motor skills, however, the oldest (61 months) was performing at 12 months below chronological age. All 6 had a gross motor delay ranging from 6 to 20 months. Our pilot data suggests proximal and distal muscle weakness in GSD IIIa and b patients. The elevated CPK in one GSD IIIb patient and muscle weakness noted on dynamometry suggest that these patients can manifest disease under stress. Further studies of genotype- phenotype correlation and effects of diet on myopathy in GSD III are currently underway.

Variable phenotypes associated with a protein truncation mutation in carnitine palmitoyltransferase II

deficiency. *G.D. Vladutiu¹, E. Quackebush², B.E. Hainline³, D. Smail¹, M.J. Bennett⁴*. 1) Dept Pediatrics, State Univ. of NY at Buffalo, Buffalo, NY; 2) Dept Neurology, Children's Hospital, Boston, MA; 3) Dept Pediatrics, Riley Hospital, Indianapolis, IN; 4) Dept Pathology, Univ. of Texas Southwestern Medical Center, Dallas, TX.

Carnitine palmitoyltransferase (CPT) II deficiency presents in 3 forms: a common adult-onset myopathy with exercise-induced pain, stiffness, and myoglobinuria, a rare lethal infantile form, and a hepatomuscular form. A single mutation, Ser113Leu, accounts for ~60% of mutant alleles in the adult disorder. We recently described a 2-bp deletion (413 delAG) in several adult compound heterozygotes. This mutation truncates the protein by one-third and is predicted to be lethal in the homozygous state. A newborn Caucasian male presented with hyperkalemia, renal cysts, and respiratory distress. Mild dysmorphic features included a high forehead, low-set posteriorly rotated ears, bilaterally undescended testes and hypoplastic toenails. Calcifications were present in the liver and cysts were found in the kidneys. Head ultrasound showed abnormalities in the parietal and frontal periventricular regions and in the basal ganglia. Lactate, pyruvate and dicarboxylic acids were elevated in the urine. The patient developed unstable cardiac function and died on the 4th day of life. CPT II activity was 18 and 28% of normal in cultured skin fibroblasts and skeletal muscle, respectively. Heterozygosity was found only for the 413 delAG mutation. A mixed race male presented at 13 months with hypoglycemia and vomiting following an 11 hr overnight fast. Physical exam was essentially normal; reduced plasma carnitine was found. While hypoglycemia and vomiting occur periodically, the patient is stable at 4 yrs of age on a low-fat diet, carnitine and MCT oil. CPT II activity in cultured fibroblasts was 18% of normal. DNA analysis revealed compound heterozygosity for the 413 delAG mutation and for Pro50His, a mutation associated with the adult form of the disease. Our findings show that the 413 delAG mutation is associated with multiple forms of CPT II deficiency and that combinations of mutant alleles appear to dictate the severity of the phenotype.

Construction and Characterization of Acid Ceramidase Deficient Mice: A New Model of Farber Disease. *J.H.*

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Acid ceramidase (AC) is the enzyme deficient in the lysosomal storage disorder, Farber Disease (FD). Features of this disorder include the progressive accumulation of ceramide and other sphingolipids leading to painful and deformed joints, subcutaneous nodules, progressive hoarseness, and a shortened life-span. Moderate neurological involvement and organomegaly also may occur. A mouse model of FD was constructed by gene targeting in ES cells. Homozygous (-/-) animals have an early embryonic lethal phenotype leading to death before day E8. Heterozygous (+/-) animals can survive up to at least one year and can reproduce, but develop marked histopathological and ultrastructural lesions similar to those reported in FD patients. The histopathology was most evident in liver, skin and cartilage, but also was observed in several other organs. Analysis of the lipids from these tissues revealed the accumulation of ceramide and several other sphingolipids. Analysis of young +/- animals prior to the onset of disease pathology also demonstrated that they may be more susceptible to radiation induced death than normal littermates. Thus, these studies reveal that the absence of AC activity in mouse embryos prevents normal development, and that heterozygosity for this knockout allele leads to histopathology and biochemical findings typical of FD.

Dicarboxylic aciduria and 3-hydroxycarboxylic aciduria in a patient of thanatophoric dysplasia type I. K.

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A thanatophoric dysplasia type I patient who survived long-term has shown abnormal urinary organic acid excretion. A full term Japanese boy, birthweight 2615g, was successfully resuscitated immediately after birth. Polyhydramnios was present. Diagnosis was made according to characteristic dysmorphic appearance and radiographic findings. Heterozygous point mutation S249C in FGFR3 confirmed the diagnosis. He needed assisted ventilation entire his life, though general condition was stable, nutritional status was stable and normal formula fed, except for last several months. He died at age of 6 years due to respiratory failure. Linear growth was very slow. Platyspondyly became less prominent in later life. Tubular bones showed almost no growth. Acanthosis nigricans was seen in later life. Non progressive ventricular enlargement, sensorineural hearing loss and neurological developmental delay were also seen. Seizure was not seen. Urinary organic acid pattern showed increased excretion of 3-hydroxybutyric acid with C6,8,10 dicarboxylic acids and 3-hydroxy C10,12,14 dicarboxylic acids. This profile suggests disturbance of β -oxidation of fatty acids leading to enhanced ω -oxidation. In addition, serum ketone bodies were increased. Various tests were carried out to determine if the underlying mechanism could be primary organic aciduria. Carnitine profiles in both serum and urine were normal pattern. CO₂ production from butyrate were normal. Very long chain fatty acid (C26:0) was not detected in serum. Thus, β -oxidation of short chain fatty acid and peroxisomal β -oxidation were not disturbed. The unusual profile could also be due to liver dysfunction and fasting, but routine laboratory test and feeding status, general condition as well altogether make these unlikely. So far, underlying biochemical mechanism of dicarboxylic aciduria is yet unknown.

Low serum creatine kinase can be seen in LGMD2H individuals with co-existing rheumatoid arthritis. *P. Frosk¹, C.R. Greenberg¹, C. Hitchon¹, J.M.G. Canvin¹, T. Weiler¹, T. Sudha¹, K. Morgan², M. Fujiwara², K. Wrogemann¹.* 1) Biochemistry and Med. Genetics, University of Manitoba, Winnipeg, Manitoba, Canada; 2) McGill University, Montreal, Quebec, Canada.

We have recently mapped a locus (*LGMD2H*), for autosomal recessive limb girdle muscular dystrophy in Manitoba Hutterites, to 9q31-33 (Weiler et al. Am. J. Hum. Genet. 63: 140-147, 1998). The assignment of clinical status in this relatively mild form of muscular dystrophy has been, at times, problematic, but we consider an individual to be affected if the muscle biopsy is dystrophic and/or the creatine kinase (CK) is at least 4 times the upper limit of normal. We recently ascertained a family with 15 offspring: Two males have classic histories of slowly progressive proximal muscle wasting and weakness, a Trendelenburg gait, myopathic EMG, and maximum recorded CK levels at 669 to 1255 U/L. One also had a clearly dystrophic muscle biopsy. We consider both to be affected. Two of their siblings had a history of seropositive rheumatoid arthritis (RA). One sibling also had signs of proximal muscle weakness and wasting, but his CK was well within the normal range of 68 to 91 U/L [normal 52-175 U/L] during the most active phase of his rheumatic disease (ESR 122 [normal 0-13 mm/h], CRP 113 [normal <8 mg/L], and RF 190 [normal <20 IU/mL]). Following treatment of his RA, his CK level rose to 898 U/L. A muscle biopsy has not yet been done. His genotypes for markers in the *LGMD2H* region are identical to his affected brothers, and we would predict his muscular dystrophy status as affected. His sister with more advanced RA has had persistently low CK levels of 30 and 35 U/L, but no clinical signs of LGMD. Her genotypes reveal a recombination in the critical region. We predict that she does not have LGMD2H. There have been previous reports of reduced CK levels associated with muscle weakness in patients with RA related to the inflammatory activity of the disease. Thus, accurate assignment of affected vs. unaffected status in muscular dystrophy families can be problematic when individuals also have rheumatoid arthritis.

Cholesterol (CH) metabolism is highly abnormal in the mouse model of Smith-Lemli-Opitz syndrome (SLOS).

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SLOS is a severe birth-defect mental retardation syndrome caused by mutations in *DHCR7*, the gene that codes for 7-dehydrocholesterol (7DHC) D7-reductase (DHCR7), the final enzyme in CH biosynthesis. As a result, plasma and tissue CH levels are abnormally reduced while concentrations of 7DHC are elevated many 100-fold. SLOS is characterized clinically by an easily recognized facies and multiple organ abnormalities.

Using heterologous recombination we mutated *Dhcr7* in the mouse. Live +/+, +/- and -/- mice were born in the ratio 1:2:1. *Dhcr7* mRNA could be identified in +/+ and +/- mice but not in -/- animals while Dhcr7 activities were found to be 206±120, 142±44 and 1±1 pmol/min/mg protein, respectively.

Homozygotes, though born live, barely moved, did not suckle, appeared bluish, breathed with difficulty and died in <1 day. Most animals had immature lungs, distended bladders, lacked chain ganglia in the thoracic cavity and many had cleft palate. Lung histology from a child with SLOS who died at 9 days looked similar to the -/- mouse.

As a result of the inhibited conversion of 7DHC to CH, CH, 7DHC and total sterol concentrations (mg/mg protein) in liver microsomes from -/- mice (17±4, 10±4, 27±8, respectively) were significantly reduced (p<0.01) compared to +/+ mice (55±11, 1±1, 56±11, respectively). Unexpectedly, in spite of low CH and total sterols, activity of HMG-CoA reductase (HMGR) was markedly reduced in the -/- compared to +/+ mice (10±8 vs. 101±75 pmol/min/mg, p<0.02) while transcription of HMGR was unchanged. However, HMGR protein levels were reduced because 7DHC caused HMGR protein to be degraded more rapidly.

The block in CH synthesis and the accumulation of 7DHC completely derange sterol metabolism in the SLOS mouse.

Simultaneous quantitation of thyroxine (T4) and thyrotropin (TSH) from newborn dried blood-spot specimens with a multiplexed fluorescent immunoassay. *R. Bellisario, R. Colinas, K. Pass.* New York State Dept of Health, Wadsworth Center, Albany, NY.

All newborns in the United States are screened by state sponsored programs for congenital hypothyroidism. Traditionally, this involved an initial screen with T4 followed by testing TSH levels of infants with low T4 values. This algorithm or an alternative whereby only TSH is assayed does not detect all hypothyroid newborns. A simultaneous assay that measures both T4 and TSH would be preferred for screening. Luminex Multi-Analyte Profiling Technology (LabMAP) was used to develop a fluorescent immunoassay measuring T4 and TSH simultaneously in a single blood spot sample. T4-BSA and anti-TSH capture antibodies were covalently coupled to two Luminex uniquely labeled fluorescent microsphere sets. Both microsphere sets were then mixed and incubated with blood spot sample extracts and biotinylated anti-T4 detection antibody for 30 min. After washing, biotinylated anti-TSH detection antibody was added and incubated for 30 min. After washing, streptavidin R-phycoerythrin was added for 15 min. The microspheres were analyzed with the Luminex100 flow analyzer. Standard curves for T4 and TSH quantitation in blood spot samples were linear over the concentrations measured (T4 : 2- 32 ug/dL; TSH : 10-160 mIU/L). The limits of detection for T4 and TSH were 0.8 ug/dL and 0.7 mIU/L respectively. The accuracy and precision of the assay method were determined using blood spot specimens from the Centers for Disease Control. For within-run imprecision, the T4 coefficient of variation ranged from 6.4% to 10.5%, and TSH from 4.2% to 5.6%. The CDC values, (targeted/observed) for T4 controls were (2.0/2.0), (5.0/4.9) and (7.5/7.4) ug/dL and (25/23), (40/36) and (80/69) mIU/L for TSH controls, respectively. Luminex technology provides a sensitive, precise, and accurate method for simultaneous measurement of T4 and TSH in dried blood spot specimens for newborn screening. It has the flexibility to add other applications to the thyroid tests, including 17-hydroxyprogesterone for congenital adrenal hyperplasia, which would multiplex the endocrinology newborn screening profile into a single test.

Mutation Analysis of Thyroid Peroxidase Gene in Taiwanese Patients with Total Iodide Organification Defects:

Identification of Five Novel Mutations. *J. Wu*^{1,2}, *C.F. Yang*^{1,2}, *F.J. Tsai*^{1,2,3}. 1) Dept Medical Research, China Medical Col Hosp, Taichung, Taiwan; 2) Dept Medical Genetics, China Medical Col Hosp, Taichung, Taiwan; 3) Dept Pediatrics, China Medical Col Hosp, Taichung, Taiwan.

Congenital hypothyroidism is one of the items that were included in the newborn-screening program in Taiwan. Its prevalence rate of one in 4,000 is second only to glucose 6-phosphate dehydrogenase deficiency. Though most cases of congenital hypothyroidism result from dysembryogenesis of the thyroid gland, about 10-20% of them are hereditary and are mostly caused by defects in the synthesis or iodination of thyroglobulin. Congenital hypothyroidism, if untreated, would inevitably lead to developmental and mental retardation; the latter is irreversible. As the successful treatment relies on an early start, the diagnosis must be established as soon as possible after birth. Thyroid peroxidase (TPO) is a key enzyme in the synthesis of thyroid hormones. It catalyzes both iodination and coupling of iodotyrosine residues in thyroglobulin. The human TPO gene is located on chromosome 2p25. It consists of 17 exons and spans about 150 kilobases in genomic DNA. To identify mutations in TPO gene, exons and exon/intron boundaries of the TPO gene were amplified by polymerase chain reaction and then screened by single strand confirmation polymorphism. PCR fragments showing aberrant shifts were then directly sequenced to identify any nucleotide changes. We identified five different mutations, all were novel. Detected TPO mutant alleles included two missense (Gly493Ser and Asp796Tyr) and three frameshift mutations (932delC, 2358insT, and 2501delC). Of those five mutations, 2358insT was most prevalent, occurring in four out of ten TPO alleles studied. The mutation study in Taiwanese patients with total iodide organification defect indicated that molecular cause for TPO mutation is highly heterogeneous and is also population specific. With most TPO mutant alleles identified, we hope to establish the genetic database for TPO mutation, which will be useful for future prenatal diagnosis and genetic counseling of congenital hypothyroidism.

Homozygosity for G530S in *COL5A1* in a Patient With the Classical Type of Ehlers-Danlos Syndrome (EDS). C.

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Skin hyperelasticity, tissue fragility with atrophic scars, and joint hypermobility are the characteristics of the classical type of EDS. The disease is usually inherited as an autosomal dominant trait, however recessive mode of inheritance has been linked to *TNX*. Mutations in the genes *COL5A1*, *COL5A2*, *COL1A1*, and *TNX* have been found in patients with classical EDS, thus confirming the suspected genetic heterogeneity. Recently, we described a patient with severe classical EDS who was compound heterozygous for two mutations in the $\alpha 1(V)$ collagen chain, the G1489E mutation in the triple-helical domain and the G530S substitution in the NH2-terminal domain, respectively. Because his affected daughter carrying only the G1489E mutation presented with a milder phenotype we inferred that G530S is a disease-modifying mutation. Accordingly, the patient's unaffected mother and daughter as well as a control individual carrying only the G530S substitution presented with thin and soft skin as the only clinical manifestation (Giunta and Steinmann, Am J Med Genet 90:72-79, 2000). Here, we report on a 4-year old boy with classical EDS, born to healthy consanguineous Turkish parents. EM revealed the typical "cauliflower" collagen fibrils in his dermis. SDS-PAGE of collagen molecules synthesized by cultured dermal fibroblasts showed normal α -chains of collagens I and III but a slightly abnormal migration of the $\alpha 1(V)$ chain. Mutation analysis on the genomic DNA and cDNA levels revealed the presence of a homozygous G530S substitution in the $\alpha 1(V)$ collagen chains and excluded an additional mutation in either the *COL5A1* and *COL5A2* genes. G530S has an incidence of 2% (2/101) and 8% (4/48) in normal Caucasian and Turkish populations, respectively. In conclusion, the findings support our previous hypothesis that the heterozygous G530S substitution is disease-aggravating and now we suggest that in the homozygous state it is disease-causing.

Clinical, mutational and enzymatic analysis of Smith-Lemli-Opitz syndrome. *P.A. Krakowiak¹, N. Javitt², C.A. Wassif¹, N.N. Nwokoro¹, F.D. Porter¹.* 1) Heritable Disorders, NICHD, National Institutes of Health, Bethesda, MD; 2) New York University Medical Center, New York, NY.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive, multiple malformation syndrome caused by mutations in the gene encoding the sterol D7-reductase. This enzyme catalyzes the reduction of 7-dehydrocholesterol (7DHC) to cholesterol in the final step of cholesterol biosynthesis via the Kandutsch-Russell pathway. The purpose of this study was to correlate detailed clinical information with molecular data including mutation and activity analysis in order to improve our understanding of the genotype/phenotype correlation of SLOS. This information will improve carrier testing, prenatal diagnosis and genetic counseling. Including mutations that we have identified, 66 different mutant alleles have been described in SLOS. To facilitate mutation analysis, we developed PCR-based restriction fragment length polymorphism (RFLP) and allele-specific oligonucleotide assays to detect five mutant alleles that account for over 60% of SLOS mutations (IVS8-1G>C, T93M, R404C, W151X and V326L). Recently, we have reported both mutant alleles in 16 SLOS patients, and we now report additional mutations in 17 SLOS patients. Identified mutations include T93M, 321G>C, L109P, T154M, I178P, T289I, R242C, A247V, Y318N, G147D, W151X, W177R, I178P, R242H, Y318N, IVS8-1G> C, V326L, L341P, R404C, Y408H, G410S, C444Y, and E448K. We hypothesized that some of the phenotypic variability observed in SLOS is due to residual enzymatic activity. In order to test this idea and to determine the biochemical severity of the different mutant alleles, we measured 7DHC and cholesterol synthesis in SLO fibroblasts by D₂O isotopic labeling. We found that cholesterol synthesis had an inverse correlation with clinical severity ($R^2=0.43$). These results suggest that although residual enzymatic activity probably accounts for some of the phenotypic variability seen in SLOS, other factors also contribute.

Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency: a novel variant of

hyperphenylalaninemia. *S. Kure*¹, *Y. Matsubara*¹, *Y. Suzuki*¹, *T. Ohura*², *K. Narisawa*¹. 1) Department of Medical Genetics, Tohoku University School of Medicine, Sendai, Japan; 2) Department of Pediatrics, Tohoku University School of Medicine, Sendai, Japan.

Hyperphenylalaninemia (HPA) is caused by a deficiency of either phenylalanine hydroxylase (PAH) or its cofactor, tetrahydrobiopterin (BH4). BH4 loading test is currently used for the differential diagnosis, because the serum phenylalanine concentration decreases after BH4 administration in BH4 deficiency, but not in PAH deficiency. Recently we encountered four patients with PAH deficiency whose elevated phenylalanine concentrations decreased in response to BH4 loading in an atypical manner. Their serum phenylalanine concentrations gradually decreased to the lowest level at 24-48 hours of administration, but never fell into the normal range (1 to 2 mg/dl). This type of response contrasted sharply with findings from patients with BH4 deficiency, in which the serum phenylalanine concentration is typically normalized within 2 to 4 hours of administration. Our patients had no abnormalities in urinary pteridine excretion or in dihydropterine reductase activity, but they had mutations in the PAH gene; patient 1, P407S/R252W; patient 2, IVS4-1G>A/A373T; patients 3 and 4, R241C/R413P (S. Kure et al., J Pediatr, 1999;135:375-8). Since patients 3 and 4 shared the same mutations, the responsiveness to BH4 appears to be determined by the nature of the PAH gene mutations. Our results suggested a novel subtype of phenylalanine hydroxylase deficiency that may be treated with cofactor supplementation. Clinical outcomes in HPA are sometimes unsatisfactory because of limited compliance with a strict phenylalanine-restricted diet. Although BH4 therapy is not always effective in PAH deficiency, it may be used as a supportive therapy for a subgroup of patients with specific PAH mutations.

Potential new mutations in the 21-hydroxylase gene causing congenital adrenal hyperplasia. *E.B. Mougey, J. Wang, R. Olney.* Dept Research, Nemours Children's Clinic, Jacksonville, FL.

The molecular genetics of 21-hydroxylase deficient congenital adrenal hyperplasia (CAH) are well described. The majority of CYP21 gene abnormalities that cause CAH are due to gene deletions or to one or more mutations transferred from the CYP21 pseudogene. Hence, screening for gene deletions and for eight common pseudogene-derived mutations will identify approximately 95% of the abnormal CYP21B genes. We used quantitative and allele-specific PCR to genotype the CYP21 gene of over thirty children with congenital adrenal hyperplasia. Here we present two previously undescribed CYP21 mutant alleles. One subject was found to have only a single copy of CYP21B. Partial sequence analysis identified a G→A mutation at nucleotide position 2163. This is the first nucleotide of intron 8 and is essential for splicing. Incorrect slicing of this intron results in a frameshift creating a premature stop codon. Several single base and frameshift mutations in the last two exons have been associated with salt-wasting CAH, demonstrating the need for these exons for normal enzyme activity. This mutation is not found in the pseudogene. Studies of the family and *in vivo* splicing analysis will be presented. Another subject was found to have a G→A mutation at nucleotide 687 resulting in replacement of Arg 102 with Lys. In addition, codon 425 is mutated to TGC from CCG thus replacing Pro 425 with Cys. The latter mutation occurs within a region of the 21-hydroxylase protein which is known to be sensitive to amino acid replacement. Neither of these mutations are found in the pseudo gene. The results of transient expression studies to determine the contribution of each of these replacements to the CAH phenotype will be presented.

Characterization of the human homologue of the yeast gene LYS5, an activator of α -aminoadipate reductase (LYS2p). *M.T. Geraghty*¹, *V. Praphanphoj*^{1,3}, *K.A. Sacksteder*², *S.J. Gould*², *G.H. Thomas*³. 1) Pediatrics; 2) Biological Chemistry, Johns Hopkins University, Baltimore, MD; 3) Kennedy-Krieger Institute, Baltimore, MD.

In mammals, L-lysine is catabolized firstly to α -aminoadipic semialdehyde by the bifunctional enzyme α -aminoadipate semialdehyde synthase and secondly to α -aminoadipate by α -aminoadipate reductase. In *S cerevisiae* the gene LYS2 encodes α -aminoadipate reductase activity while a second gene LYS5 encodes a phosphopantetheinyl transferase, required to activate LYS2p. Using the BLAST algorithm and 5' RACE we cloned a full-length human cDNA homologous to the yeast LYS5 gene. The cDNA contains an ORF of 927 bp predicted to encode 309 amino acids. The human protein is 26% identical to its yeast counterpart. In Northern blot analysis the cDNA crosshybridizes to a single transcript of approximately 3kb in all tissues except testis, where there is an additional transcript of 1.5kb. Expression is highest in brain followed by heart and skeletal muscle and surprisingly to a lesser extent in liver. We further identified 3 human genomic DNA BAC clones containing the LYS5 gene sequence. FISH analysis using the BAC clones mapped the gene to chromosome 11q22 while alignment of the cDNA and genomic DNA sequences allowed partial identification of the intron-exon boundaries. Finally, using one-step homologous recombination in *S cerevisiae* we generated a *lys5* knockout strain. Complementation studies with normal and mutated human LYS5 cDNAs in a yeast expression vector have shown that the human homolog functions similarly to the yeast LYS5. We hypothesize that defects in this gene may result in hyperlysinemia, saccharopinuria or pipecolic aciduria.

Biochemical characterization of SRD5A2 gene polymorphisms found in Benign Prostatic Hyperplasia (BPH) patients. *N.M. Makridakis, J.K.V. Reichardt.* Biochem/Mol Biol/Inst Gen Med, Univ Southern California, Los Angeles, CA.

Both BPH and prostate cancer are prevalent diseases of mainly unknown etiology that are believed to be androgen dependent. The SRD5A2 gene encodes the prostatic steroid 5 α -reductase (type 2), which converts testosterone into dihydrotestosterone (DHT), the major androgen in the prostate gland. We have recently shown that the SRD5A2 gene is involved in prostate cancer predisposition. We then set out to examine the hypothesis that specific SRD5A2 mutations predispose men to develop BPH, through increased androgen metabolism. We screened the genomic sequence of the SRD5A2 gene for polymorphisms with direct sequencing analysis. We utilized lymphocyte DNA from mostly Caucasian-American patients whose prostate glands were evaluated for the presence of BPH or prostate cancer, after bladder cancer surgery. We report the finding of 14 polymorphisms in the BPH samples, 12 novel ones, and two previously identified polymorphisms. Three of those novel polymorphisms are relatively common. The most common polymorphism, C682G (cytosine-682 to guanine), is located in the 5' UTR (untranslated region) and is significantly more common in men with early-onset BPH. In order to characterize the biochemical effect of some of those polymorphisms, we have subcloned a 750-bp fragment containing the promoter and 5' UTR of the SRD5A2 gene in front of the luciferase gene on the pGL3 expression vector. After appropriate site-directed mutagenesis construction, we transfected *cos* cells with either the wild type (682C) or the mutant (682G) construct, and measured the luciferase activity of the isolated transfected cell lysates. The C682G polymorphism resulted in significantly higher luciferase expression, suggesting that it may predispose people to develop BPH earlier, through increased 5 α -reductase enzyme levels. In conclusion, so far we have evidence that the SRD5A2 gene is polymorphic in BPH patients, and that at least one of these polymorphisms (the C682G) may increase 5 α -reductase enzyme levels in vivo.

T cell dependent immune response in a knockout model of lysosomal storage disease. *K. Nagaraju¹, N. Raben¹, E. Lee¹, P. Rochon¹, N. Lu¹, G. Yap², S. Manetz¹, P. Plotz¹.* 1) ARB, NIAMS, NIH, Bethesda, MD; 2) NIAID, NIH, Bethesda, MD.

Acid α -glucosidase is a lysosomal enzyme which degrades glycogen. The deficiency of this enzyme leads to glycogen storage disease type II (GSD II). We have previously generated a mouse model of GSD II by targeted disruption of the acid α -glucosidase gene (GAA^{-/-}). The knockout mice accumulate lysosomal glycogen in multiple organs and develop clinical symptoms similar to those in humans with GSDII. We found abnormally enlarged lysosomes with glycogen accumulation in the peritoneal macrophages of the GAA^{-/-} mice (ko) but not in the wild type (wt) littermates. Since intact functional lysosomes are important for effective immune responses, we have investigated the ability of these mice to mount an immune response to a known T cell dependent antigen, nitrophenylacetyl-keyhole limpet hemocyanin, NP-KLH. After immunization with NP-KLH, the ko mice showed reduced levels of NP-KLH induced IL-4 compared to wt littermates as measured by ELISA (ko vs wt, mean \pm se, pg/ml: 12.7 \pm 4.7 vs 47.7 \pm 18.3), suggesting a weaker Th2 response in the ko mice. However, the knockouts mounted a stronger Th1 response as indicated by the levels of NP-KLH induced IFN- γ production (ko vs wt, mean \pm se, pg/ml: 672 \pm 452 vs 307 \pm 240). Furthermore, we have demonstrated that the macrophages from mutant and wt mice exhibit a comparable response to both lysosomal dependent (*Listeria*; CFU counts: ko vs wt, mean \pm se, 129500 \pm 26180 vs 95000 \pm 12096) and independent (*Toxoplasma*; killing: ko vs wt, mean \pm se, 100 \pm 0.6 vs 103 \pm 1.1) microorganisms. These results demonstrate that despite profound lysosomal damage, the ko mice can mount an adequate Th1 response to a T cell dependent antigen and effectively react to intracellular pathogens, indicating that newly formed lysosomes or non-lysosomal pathways may be involved in the immune response.

Spectrum of mutations in *SLC17A5* gene resulting in free sialic acid storage diseases indicates some genotype-phenotype correlation. *N. Aula*¹, *P. Salomäki*², *R. Timonen*¹, *F. Verheijen*³, *G. Mancini*³, *J-E. Månsson*⁵, *P. Aula*⁴, *L. Peltonen*^{1,4}. 1) Human Molecular Genetics, National Public Health Institute, Helsinki, Finland; 2) Department of Medical Genetics, University of Turku, Finland; 3) Department of Clinical Genetics, Erasmus University and Academic Hospital, Erasmus Medical Centre of Rotterdam, The Netherlands; 4) Department of Medical Genetics, University of Helsinki, Finland; 5) Department of Psychiatry and Neurochemistry, University of Göteborg, Mölndahl, Sweden.

Lysosomal free sialic acid storage diseases include allelic disorders Salla disease (SD) and infantile sialic acid storage disease (ISSD). The gene, *SLC17A5*, coding for the lysosomal free sialic acid transporter was recently isolated. In the present study we have identified a large number of mutations in *SLC17A5* in patients presenting either Salla disease or ISSD phenotype. All Finnish SD patients (n=80) had a missense mutation changing a highly conserved arginine to cysteine (R39C), 91% of them being homozygous for this old founder mutation. The compound heterozygote patients with the founder mutation in only one allele presented with a more severe phenotype than the homozygote patients. The same R39C mutation was also found in most of the Swedish SD patients and in a heterozygous form in five patients from central Europe presenting an unusually severe (intermediate) phenotype. Ten different mutations including deletions, insertions, missense and nonsense mutations were identified in patients with the most severe, ISSD phenotype, most being compound heterozygotes. Our results indicate some genotype-phenotype correlation in free sialic acid storage diseases suggesting a milder phenotype to be associated with the homozygote R39C mutation than with other mutations.

Six novel mutations in the alpha-galactosidase A gene with classical phenotype. *K. Azibi^{1,2}, C. Caillaud^{1,2}, J. Manicom¹, J.P. Puech¹, A. Kahn², L. Poenaru^{1,2}.* 1) Biochimie Genetique, Faculte de Medacine Cochin, Paris, France; 2) INSERM U-129, CHU Cochin, Paris, France.

Fabry disease is an X-linked glycosphingolipid storage disease resulting from deficient activity of the lysosomal alpha-galactosidase A (GLA). This deficiency leads to the accumulation of uncleaved glycosphingolipids in the lysosomes of vascular endothelial and smooth muscle cells and also in plasma. The clinical manifestations in classical form are due to a small vessels pathology resulting in angiokeratoma, renal failure and heart and brain ischemia. Death occurs from renal or cardiac complications. There are also atypical forms with residual GLA activity and late-onset cardiomyopathy. Patients studied have a classical Fabry disease, with angiokeratoma, abdominal pain, corneal opacity, paresthesias in the extremities and renal failure. One patient presents a dementia. In one family with three patients, one was a female with a polycystic kidney. The alpha-galactosidase was deficient in all these patients with a residual activity between 0,8 to 4 nmol/h/mg protein. To clarify the molecular mechanism causing the enzyme defect and to facilitate a rapid detection of hemizygote and carrier, we used a direct sequencing strategy to determine the GLA gene alterations. The seven exons including intron-exon junctions were PCR amplified and the products directly sequenced. Six novel mutations were identified, including two nonsense mutations (W245X and W262X) and two missense mutations : A156N (G-->C transition generating a novel SfaNI restriction site) and Q279H (C-->A transition creating a novel HphI site). A small nucleotide insertion (InsG10678) was also detected, generating a codon stop at 332 on the cDNA, as well as a small deletion (1235del15) which creates a novel Sau96I restriction site and obliterates a HaeIII site. The affected female carries the novel 1235del15 in the GLA gene. Her clinical expression is probably due to the extreme lyonisation of the normal X-chromosome. Our study further defines the previously reported heterogeneity of mutations causing the disease. It has also permitted to clarify the carrier status and to facilitate the prenatal diagnosis.

Mutation analysis in patients with human b-mannosidosis. *R. Bedilu, K.A. Nummy, K.H. Friderici.* Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI.

b-Mannosidosis is a lysosomal storage disease with autosomal recessive inheritance resulting from a deficiency of the lysosomal enzyme b-mannosidase. The clinical manifestations of this disease in human cases reported to date are relatively mild for lysosomal storage disease and rather heterogeneous in nature. Reported patients have exhibited variable and usually mild clinical presentation compared with the severe prenatal onset seen in ruminant b-mannosidosis. In humans, retardation, hearing loss, frequent infections, and behavioral problems are relatively common. Dismorphology and skeletal involvement such as that seen in ruminants are rare. In this study, mutations associated with two confirmed patients with disaccharide storage were determined using genomic DNA isolated from patients' cultured skin fibroblasts. We determined that the b-mannosidase gene consists of 17 exons. Intron based PCR primers were designed and used to amplify each of the exons. The PCR products were purified, directly sequenced, and analyzed for sequence variation by comparison with the normal sequence. Results of the analysis showed that one patient was homozygous for a missense mutation G632A in exon four. This missense sequence variation in the coding region of b-mannosidase was compared to that of goat, bovine, and mouse sequences. It is predicted to affect enzyme function because it results in an amino acid substitution R182Q producing a change in charge in a conserved region. A second patient was homozygous for a nonsense mutation due to two base pair deletion (AT) in exon 11 at positions 1541 and 1542 of the cDNA sequence. This deletion causes a reading frame shift and 26 out of frame amino acids before a stop codon occurs in exon 12 resulting in truncation of the deduced peptide sequence from 879 to 510 amino acids. Because disease presentation in reported patients is relatively mild, the null mutation found in this study leads us to conclude that b-mannosidosis in humans is indeed mild compared to ruminant disease. Studies to identify mutations in additional confirmed patients, and the structure-function relationships of b-mannosidase are underway.

Characterization of a pseudogene homologous to the UDP-N-acetylglucosamine 2-epimerase gene; relevance for mutation detection in patients with sialuria. *M. Huizing, Y. Anikster, W.A. Gahl.* Section on Human Biochemical Genetics, Heritable Disorders Branch, NICHD, NIH, Bethesda, Maryland 20892.

Sialuria is a rare inborn error of metabolism characterized by cytoplasmic accumulation and increased urinary excretion of free N-acetylneuraminic acid (sialic acid). Overproduction of sialic acid results from loss of feedback inhibition of the rate-limiting enzyme in sialic acid synthesis, UDP-GlcNAc 2-epimerase, by the activated sialic acid donor cytidine monophosphate-N-acetylneuraminic acid (CMP-sialic acid). All 4 of the sialuria patients examined to date have had heterozygous mutations in the UDP-GlcNAc 2-epimerase gene corresponding to the allosteric site of the epimerase; the specific mutations were R263L, R266W and R266Q. Here we report a pseudogene, partially homologous to the UDP-GlcNAc 2-epimerase gene, whose allosteric region contains the mutant codon CAG (normal, CGG) corresponding to the amino acid change R266Q. Several points follow from this finding: 1. The mutation represents a typical CpG dinucleotide transition from CG to TG (antisense). 2. The allosteric region of the gene might be subject to a high mutation rate, but in the transcriptionally active gene, the DNA repair mechanism readily corrects mutations. In the pseudogene, however, the repair mechanism functions poorly, and does not correct the mutation. 3. Mutation analyses of sialuria patients at the gDNA level will be complicated by PCR coamplification of pseudogene sequences. It is therefore recommended to screen with primers located in introns or to screen at the cDNA level. Recognition of the existence of this pseudogene may help prevent misinterpretation of mutation analyses in sialuria patients.

Generation of a transgenic mouse with a polymorphic change in the GALC gene to investigate clinical variability in Krabbe disease. *P. Luzi¹, M.A. Rafi¹, J.S. Khillan², M. Zaka¹, M.T. Vanier³, M.T. Curtis⁴, D.A. Wenger¹.* 1) Neurology, Jefferson Medical Col, Philadelphia, PA; 2) Wistar Institute, Philadelphia, PA; 3) INSERM U189, Oullins, France; 4) Pathology, Jefferson Medical Col,.

Krabbe disease (KD) or globoid cell leukodystrophy is caused by mutations in the galactocerebrosidase (GALC) gene. This lysosomal enzyme is responsible for the degradation of specific galactolipids found mostly in myelin. While 85-90% of the human patients have the severe infantile form, older patients and adults are also diagnosed. All patients have very low (0-5% of normal) GALC activity. There is no significant difference in residual activity between the infants and adults. About 60 disease-causing mutations and four polymorphisms have been identified. In our screen of patients with undiagnosed neurological disease, individuals have been identified with GALC activity 15-25% of our normal mean. This is clearly above the activities found in confirmed cases of KD. DNA analysis reveals that all of these individuals have multiple polymorphic changes in the two GALC alleles. It is proposed that such low GALC activity might cause disease if certain environmental and/or genetic components also were present. In order to obtain experimental evidence for this, transgenic mice with a common human polymorphism, arg to cys at amino acid position 168, were generated. In vitro expression studies demonstrated that this amino acid change in the mouse protein resulted in about 10-20% of normal activity. Heterozygous mice were produced and they had about 50-60% of normal GALC activity in all organs. Homozygous mice were recently born and all organs showed the expected low GALC activity. At 30 days of age these mice are maintaining their weight and show no sign of twitching, as always occurs in twitcher mice of this age. The brain and other organs will be examined pathologically. Homozygous mice will be subjected to chemical and viral insults to induce demyelination. It is proposed that these mice will not recover as normal mice do because of the sub-optimal GALC activity. This model also may be useful to explain the wide range of clinical onset observed in the milder forms of KD.

Gene Disruption of the CTNS Homologue in Yeast Does Not Result in Intracellular Cystine Storage. A. Helip Wooley, J. Thoene, L. Hyman. Hayward Genetics Center, Tulane University School of Medicine, New Orleans, LA., USA.

Cystinosis is caused by defective transport of the disulfide amino acid cystine out of the lysosome, resulting in intralysosomal cystine storage. The gene responsible for cystinosis, CTNS, encodes a 367 amino acid protein that has 43% identity and 64% similarity over 102 amino acids with the yeast transmembrane protein Ers1. The function of the Ers1 gene product is unknown, but the acidic vacuole of yeast (a lysosome analog) possess a large pool of reduced glutathione, which should preclude the existence of cystine in this compartment and perhaps obviate the need for a transporter. A viable Ers1 gene knockout yeast strain was created from *Saccharomyces cerevisiae* strain W303 using a kanamycin gene disruption cassette. The intracellular cystine of wild-type and DErs1 yeast, as measured by a cystine binding protein assay, did not differ. No differences in intracellular glutathione concentrations were observed. Amino acid analysis by HPLC, organic acids by GC-MS and mono- and di-saccharides by TLC also showed no differences between the two strains. Despite sequence homology, Ers1 and CTNS protein products do not appear to have homologous function, as no substrate for known lysosomal transporters accumulated in the yeast acidic vacuole in the absence of Ers1. Further investigations are needed to identify the function of Ers1, the similarity of Ers1 and CTNS proteins and how yeast acidic vacuoles differ from human lysosomes.

Correction of the Cystinosis Phenotype in Cultured Cells by an Aminoglycoside. *R. Lemons, A. Helip Wooley, J. Thoene.* Hayward Genetics Center, Tulane University School of Medicine, New Orleans, LA.

Aminoglycoside antibiotics cause misreading of termination codons in mRNA resulting in read-through of premature stop codons caused by nonsense mutations. Fibroblasts derived from patients with known mutations in the CTNS gene were exposed to gentamicin at a concentration of 300 mg/ml for periods of up to 15 days. The cells were harvested at different time points, and the intracellular cystine content was measured by a cystine binding protein assay. Cells heterozygous for a premature termination mutation (753 G ® A) and a splice site mutation (11 IVS +2 T ® C) incubated in the presence of gentamicin demonstrated between 53% (after 5 days) and 24% (after 15 days) of the cystine content of control cells not treated with gentamicin ($p < 0.001$ d 15). Cells homozygous for a 57-kb deletion, including most of the CTNS gene, showed no decline in cystine (101% of control); and cells homozygous for a 5-bp deletion (545 del TCCTT) demonstrated increased cystine (199% of control). Cystine depletion resulting from aminoglycoside treatment was specific to cell strains possessing a premature termination codon mutation, was not seen prior to 5 days, and was maintained through day 15. Aminoglycosides are known to be nephrotoxic, making this an unlikely treatment for a nephropathic disease like cystinosis. Nevertheless, these results are intriguing and suggest the need for further investigation into this class of compounds as a way to alter gene expression in patients with premature termination codons resulting in disease.

Clinical and genetic heterogeneity in GM1 gangliosidosis patients. *A. Morrone¹, T. Bardelli¹, A. Caciotti¹, R. Gallo¹, S. Bisanzi¹, M.A. Donati¹, A. D'Azzo².* 1) Dept of Paediatrics, Florence, Italy; 2) Dept. of Genetics, St. Jude Hospital, Memphis, USA.

GM1-gangliosidosis is an AR lysosomal storage disorder caused by deficiency of acid -galactosidase (GLB1). The disease has been classified into three clinical forms: infantile, late infantile or juvenile, and adult or chronic. The severe infantile form shows rapidly progressive CNS involvement, facial and skeletal abnormalities, and hepatosplenomegaly. Cardiomyopathy can be associated with this phenotype. The juvenile form is characterised by slowly progressive psychomotor deterioration. The rare adult form is specified by mild clinical manifestations. The GLB1 gene gives rise to two alternately spliced mRNAs: a major transcript which encodes the lysosomal enzyme and a minor transcript encoding the Elastin Binding Protein (EBP), a major component of the non-integrin cell surface receptor complex. We report the identification of five new GLB1 mutations in the total RNA and DNA preparation of 4 Italian GM1 gangliosidosis patients. Two new aminoacid substitutions S54N/R59C were detected in one infantile patient with cardiomyopathy. The known G579D and an aberrant transcript devoid of Exon 2 were detected in one infantile patient. A juvenile patient was compound heterozygous for the new C230Y and the known R201H. Two new mutations T329A/R442Q were detected in an adult patient. Some of the genetic lesions detected in the patients' GLB1 gene were transiently expressed in COS1 cells. No GLB1 activity was detected for the aminoacid substitutions in the infantile patients and for the T329A present in the adult, demonstrating the deleterious nature of these defects. The R442Q showed a residual enzymatic activity of 10% and correlated with the adult phenotype. Both genetic lesions detected in the juvenile patient are localised in a region of pre-mRNA encoding for only the lysosomal enzyme. The other genetic lesions affect both the GLB1 and EBP cDNA and these proteins, altered in function and/or distribution, contribute differently to the specific clinical manifestations. The financial support of Telethon-Italy (Grant n.E.852) and Associazione Italiana MPS are gratefully acknowledged.

1bp deletion in saposin B domain of the prosaposin gene leads to nonsense-mediated mRNA decay and prosaposin deficiency. *M. Håebick, M. Èervenková, H. Hùlková, M. Tocháèková, J. Ledvinová, H. Poupìtová, M. Elleder.* Inst. Inherited Metab. Dis, 1.stFaculty of Medicine, Ke Karlovu 2, Praha 2, Czech Republic.

We present a patient with generalised lysosomal sphingolipid storage and immunohistochemical evidence of saposin A, B, C, and D deficiency. The patient presented at birth with hepatosplenomegaly and generalised seizures. The patient died at 4 months of age due to progressive generalised storage disorder. Storage macrophages were found many organs at post-mortem. Gross elevation of glucosylceramide, globotriaosylceramide and lactosylceramide and moderate elevation of ceramide was found in liver and spleen. Sulfatides and galactosylceramide were highly elevated in the kidney. Cholesterol and sphingomyelin concentrations were not elevated. Immunohistochemical analysis with antibodies raised against saposins A, D and saposin C showed the absence of immunoreactive material in all studied tissues. The patient was found to be homozygous for a single base deletion in exon 9 (c803delG) by sequencing of PCR products from genomic DNA. The deletion leads to a frameshift and after 9 amino acids to a premature stop codon. No other nucleotide changes were found in the sequenced parts of the gene. The c803delG, which is located in the saposin B domain is apparently a null mutation, as the frameshift causes premature stop codon, likely to cause nonsense-mediated mRNA decay. In the heterozygous parents direct sequencing of RT-PCR products revealed only the wild-type cDNA sequence, suggesting that the mutant mRNA was significantly less abundant. Mutant cDNA was detected by ARMS only in RNA isolated from nuclear fraction of parents fibroblasts, it was, however, not detectable in the cytoplasmic fraction. This confirms that the mutant mRNA is in the cytoplasm essentially absent or at least significantly less abundant - a finding compatible with the nonsense-mediated decay of mutant mRNA in the nucleus. These findings together with the lack of immunoreactive saposins A-D in the patients tissues allow to postulate that the c803delG in addition to deficit of saposins B,C, and D leads also to a functional deficit of saposin A due to nonsense-mediated decay.

Model for Kupffer cell proliferation in lysosomal acid lipase deficient mice. *M. Heur¹, H. Du¹, D. Witte², G.A. Grabowski¹*. 1) Div Human Genetics, Children's Hosp Med Ctr, Cincinnati, OH; 2) Div Pathology, Children's Hosp Med Ctr, Cincinnati, OH.

Lysosomal acid lipase (LAL) cleaves dietary cholesteryl esters (CE) and triglycerides (TG) that are delivered to the lysosomes. A major characteristic of LAL deficient mice (*lal*^{-/-}) is the massive hepatomegaly (< 40% of body weight) with progressive lysosomal storage of CE and TG. Initial hepatocellular storage is replaced by massive hypertrophy and hyperplasia of Kupffer cells that are arranged into discrete, clonal proliferative foci. The objective of this study was to define the etiology of Kupffer cell proliferation in *lal*^{-/-} mice. Ribonuclease protection assays (RPA) showed a 14.4-fold increase of M-CSF mRNA in 2 mos. old livers of *lal*^{-/-} vs. *lal*^{+/+} mice. Other cytokines examined by RPA were IL3, IL11, IL7, GM-CSF, G-CSF, LIF, IL6, and SCF, but none of their messages were detected in *lal*^{-/-} or *lal*^{+/+} livers. In situ hybridization of 1, 4, and 8 mos. old liver sections from *lal*^{-/-} mice showed similar levels of M-CSF mRNA in hepatocytes. Very weak signals were present in the Kupffer cells at 1 mo., but with increasing age, Kupffer cell signals became stronger so that by 8 mos., Kupffer cell and hepatocyte signal strengths were similar. Niemann Pick C (*npc*^{-/-}) mice have a spontaneous mutation in a protein transporter leading to deficient free cholesterol (FC) egress from the lysosome. Thus, *npc*^{-/-} cells accumulate FC in the lysosome, but lysosomal egress of free fatty acid is not affected. Livers of *npc*^{-/-} mice show hepatocyte storage of FC, but Kupffer cells are not the major storage cells. RPA of 2 mos. old livers showed that M-CSF mRNA levels were elevated 3.5-fold in *npc*^{-/-} vs. *lal*^{+/+} mice and 13-fold in *lal*^{-/-}, *npc*^{-/-} vs. *lal*^{+/+} mice. This indicates a model in which tonic suppression of M-CSF expression in hepatocytes is regulated by lysosomal egress of CE and TG derived lipids.

Proteomic maps of lysosomal membranes as a tool to study the molecular basis of lysosomal transport diseases.

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Lysosomal membrane transport proteins mediate the traffic of protons, cysteine or activated acetyl residues into the lysosome and the catabolic products such as amino acids, monosaccharides and vitamins out of the lysosome. Lack or malfunction of lysosomal transporter proteins causes several severe genetic diseases of children including nephropathic cystinosis (CTNS), Niemann-Pick disease type C, sialic acid storage disease (SIASD), cobalamin (cbl) F disease, mucopolidosis IV (ML IV) and Sanfilippo III type C (MPS IIIC). The molecular mechanisms of three last diseases have not been yet clarified. The goal of our proteomic-based research program is to identify the underlying cause of genetic diseases, as reflected by the protein patterns of affected cells and tissues. We have isolated lysosomal membranes from mouse and human liver, obtained their proteomic maps using two-dimensional gel electrophoresis with immobilized pH gradients and identified the major proteins by combining peptide mass fingerprinting, and amino acid sequencing. The obtained results have provided the basis to build a complete proteomic database of the lysosomal membrane. Then we have analyzed lysosomal membrane proteins from cultured skin fibroblasts of normal controls and patients suffering from Salla disease, cbl F disease, CTNS, and MPS IIIC and identified the protein spots that reproducibly differ between patients and control cells. Candidate disease-causing genes will be cloned, and studied by mutation analysis and/or by expression and functional characterization. This approach may be in the future extended to discovery of other disease-related membrane proteins in peroxisomes, microsomes, mitochondria and the plasma membranes.

Gaucher disease type 2 presents as non-immune hydrops and hypokinesia. *L.H. Seaver¹, N.R. Cooley, Jr.¹, R.S. Colby¹, E. Boyd².* 1) Greenwood Genetic Center, Greenwood, SC; 2) Fullerton Genetics Center, Asheville, NC.

The development of a null-allele knockout Gaucher disease mouse model has led to increased recognition of a human perinatal lethal form of Gaucher disease type 2. Phenotypic heterogeneity is demonstrated by an ectodermal phenotype ("collodion" baby, ichthyosis, restrictive dermopathy) and a non-immune hydrops (NIH) phenotype. We report three consecutive pregnancies affected with Gaucher disease born to a non-consanguineous Caucasian couple. NIH was identified in each affected fetus and presented after 20 weeks gestation. In addition, at autopsy, each showed evidence of intrauterine hypokinesia with contractures of large and small joints, and decreased flexion creases. Intrauterine growth retardation and hepatosplenomegaly were also identified. Ectodermal changes were not obvious, possibly due to maceration of tissue. Intrauterine fetal demise was diagnosed at 28-30 weeks gestation in each pregnancy. The third pregnancy was identified as abnormal at 20 weeks gestation because of striking hypokinesia noted on ultrasonography, which predated the development of NIH. The diagnosis was made in the third affected fetus with very low b-glucosidase activity. We hypothesize that fetal hypokinesia may be the result of the neurotoxic compound glucosylsphingosine, which is elevated in both the mouse model and patients with Gaucher disease type 2. Ectodermal changes may also contribute to hypokinesia. A variety of mechanisms are likely to lead to NIH in Gaucher disease and other lysosomal storage disorders.

The NIH presentation of lysosomal storage disorders is frequently not recognized or diagnosed prenatally, especially in a first affected pregnancy. Increased recognition of these frequently lethal lysosomal storage disorders should lead to enzymatic and/or molecular diagnosis that may change obstetric management decisions, and also allow early prenatal diagnosis in subsequent pregnancies.

The Association of Hereditary Hemochromatosis (HH) and Gaucher's Disease (GD): A Case Report. *A. Prakash-Cheng¹, Q.-Y. Li¹, J.A. Strauchen², M.E. Grace¹, P.K. Mistry¹*. 1) Dept Human Genetics, Mount Sinai School of Medicine, NY, NY; 2) Dept Pathology, Mount Sinai School of Medicine, NY, NY.

Hyperferritinemia with or without elevated transferrin saturation is frequently found in patients with type I GD, as evidenced by abundant iron accumulation in Gaucher cells. This is generally ascribed to an acute phase reaction that accompanies macrophage activation in GD. We report a family with type I GD and HH.

AV is a 44 year old woman of Ashkenazi Jewish ancestry who presented with chronic fatigue, hepatosplenomegaly, thrombocytopenia and elevated serum ferritin. A liver biopsy revealed grade 3+ hepatocyte siderosis and iron accumulation in Kupffer cells. MRI was consistent with iron overload in the liver. Bone marrow aspirate revealed Gaucher cells. DNA analysis revealed homozygosity for both N370S mutation at the *GBA* locus and H63D mutation at the *HFE* locus. Her hematological indices improved and splenomegaly regressed following enzyme replacement therapy with recombinant acid b-glucosidase (Cerezyme®). Subsequently, monthly phlebotomy was commenced, requiring 20 units of venesection to normalize her serum iron indices. Significant reduction of hepatic iron overload was demonstrated by MRI. Patient AV is one of three siblings. Her sister died at age 40 following surgery for a presumed pituitary adenoma. Histology of the surgical specimen revealed lymphocytic hypophysitis, but no pituitary adenoma. Post-operatively, she developed meningitis and hepatic and bone marrow dysfunction. She died of multi-organ failure. DNA analysis of pituitary tissue from a paraffin block revealed homozygosity for the H63D mutation. Analysis of the *GBA* locus is in progress. Review of bone marrow aspirate revealed Gaucher cells.

This case report raises the possibility that the abnormality of iron metabolism in some patients with GD may be associated with the presence of the H63D mutation. Studies are underway in a large cohort of patients with type I GD and elevated serum ferritin to determine the prevalence of the *HFE* mutations and correlate these with abnormal iron indices and evidence of tissue iron overload.

W381Ter: Novel mutation in the coding sequence of the acid b-glucosidase gene of a Type 1 Gaucher patient who developed neutralizing antibodies. *E. Ponce, G.A. Grabowski.* Division of Human Genetics, Children's Hospital Medical Center, Cincinnati, OH.

Numerous gene sequence variations of the enzyme acid b-glucosidase (GBA) have been associated with Gaucher disease (GD), an inherited glycosphingolipid storage disorder resulting from decreased activity of this lysosomal enzyme. Causal to the manifestations of this disease is the accumulation of unprocessed glucocerebrosides and other metabolic pathway byproducts in the lysosomes of cells of monocyte/macrophage origin of organs involved. These result in altered cell physiology leading to hepatosplenomegaly, hypersplenism, bone disease and CNS manifestations. GD patients are commonly diagnosed by verification of deficient GBA activity in lymphocytes and partial genetic characterization. The latter is based on the identification of the most common alleles namely N370S, L444P, IVS2+1 and 84GG by using PCR-based methods. In order to further explain structure/function of GBA and genotype/phenotype correlations in GD patients, we have undertaken the identification of novel alleles by direct sequence analysis of the GBA locus. Bidirectional sequencing was obtained by genomic DNA PCR amplification, followed by capillary electrophoresis analysis of Dye terminator sequencing reactions of the amplicons, and comparison to the GenBank human GBA gene sequence (accession # J03059). In addition to several single nucleotide polymorphic variants, we have identified a W381Ter novel mutation in a type 1 patient receiving enzyme replacement therapy for severe GD, in which only an N370S allele had been previously reported. This patient required several therapeutic approaches, including a tolerization protocol, due to the development of neutralizing antibodies during treatment. Identification of this stop codon mutation may help understanding the severity of the disease in the presence of a quote "milder allele" and the role for the C-terminus of the enzyme.

Carrier frequency of the common mutation IVS8-1G>C in *DHCR7* and estimate of the expected incidence of Smith-Lemli-Opitz syndrome. *K.P. Battaile¹, B.C. Battaile⁴, C. Maslen², R.D. Steiner^{1,3}.* 1) Department of Pediatrics, Oregon Health Sciences University, Portland, OR; 2) Department of Medicine, Oregon Health Sciences University, Portland, OR; 3) Department of Molecular and Medical Genetics, Oregon Health Sciences University, Portland, OR; 4) School of Fisheries and Ocean Sciences, Juneau Center, University of Alaska Fairbanks, Juneau, AK.

Smith-Lemli-Opitz syndrome (SLOS) is a multiple congenital anomaly/mental retardation syndrome of variable severity with an incidence previously estimated at 1 in 20,000-60,000 based on case frequency surveys. Identification of the gene defect in SLOS has made it possible to calculate the carrier frequency and estimate disease incidence using molecular methods to identify carriers. Using a previously described PCR-RFLP assay we screened 1503 anonymous blood samples from random newborn screening blood spot cards for the presence of the common SLOS mutation IVS8-1G>C in order to determine the carrier frequency. 16 carriers were identified in the 1503 samples. Since the frequency of the IVS8-1G>C mutation among all SLOS gene mutations is known, the overall carrier frequency for all mutations can be calculated. The calculated carrier frequency for all mutations based on this result is 1 in 30, predicting an SLOS incidence of 1 in 1773 to 1 in 10,204. The current incidence estimate may, therefore, significantly underestimate the true incidence of SLOS. This discrepancy between calculated and observed incidence could be due to undiagnosed mild cases, misdiagnosed severe cases, death prior to diagnosis, or fetal loss. Determining the actual incidence of SLOS will be important for policy decisions such as prioritizing the development of universal newborn screening and universal prenatal screening for SLOS, particularly if treatment can improve the outcome of patients with SLOS. More comprehensive incidence studies are needed to determine if SLOS is as common as predicted by the very high (1 in 30) carrier frequency determined in this study.

HMG-CoA Lyase deficiency in an Italian patient is caused by a new nonsense mutation in HMGCL gene.. S. Funghini, E. Pasquini, M.A. Donati, A. Morrone, E. Zammarchi. Dept of Paediatrics, "A. Meyer" Children Hospital, Florence, Italy.

3-OH-3-methylglutaric aciduria is a rare A.R. inborn error of metabolism due to the deficiency of the mitochondrial enzyme HMG-CoA lyase that catalyses the cleavage of HMG-CoA to acetoacetate and acetyl-CoA. The disease has a neonatal or postnatal onset. Clinical acute episodes are characterized by vomiting, hypotonia, lethargy and coma. Metabolic acidosis with hypoketotic hypoglycemia on fasting, variable hyperammonaemia and high plasma transaminase levels may be present. The patients show a characteristic organic aciduria: 3-OH-3-methylglutaric acid, 3-methylglutaconic acid, 3-methylglutaric acid, and 3-OH-isovaleric acid. HMGCL gene, mapped on 1p36.11, has been cloned. Up to now only few mutations have been reported and most of them were identified in Arabic origin patients. We report an Italian female patient born at 40th week following an uneventful pregnancy and delivery. At three days of life she showed metabolic acidosis: renal tubular acidosis was diagnosed. At three months she was admitted to our hospital for vomiting and lethargy; hepatomegaly and hypotonia were present. Laboratory investigations revealed metabolic acidosis, increased anion Gap, hypoglycaemia, hyperlactacidemia, hypertransaminasemia, macrocytic anemia and low levels of total and free carnitine. Brain MRI revealed an increased signal of periventricular white matter. Analysis of urinary organic acid by GC/MS showed the characteristic pattern of HMG-CoA lyase deficiency. On protein-leucine-restricted diet, low fat and L-carnitine supplementation, the concentration of pathological urinary organic acids decreased markedly. Hepatomegaly disappeared in one month and the transaminases normalized after two months. Fibroblast HMG-CoA lyase activity was 0.33kat/kg/prot(n.v.18.1± 6.9). Molecular analysis of full length patient's HMGCL cDNA showed a new c286C>T transition that leads to the Q96X stop codon. This mutation detected at homozygous level was confirmed in the patient's and parents genomic DNA. The Q96X gives rise to a truncated protein in which a high percentage of the amino acid sequence is lost, including the domain that contains the active catalytic site.

Cobalamin (Cbl) deficiency: presentation, complications, long-term treatment and outcome in 5 children. *G.T. Berry, A. Laufer-Cahana, C. Venditti, M. Miccolis, M. Harrington, P. Thornton, E.M. Kaye, P. Kaplan.* Genetics Division, Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, PA.

Cbl C deficiency is characterized by inability to convert cbl to methyl cbl and adenosyl cbl, with resulting excessive methylmalonic acid and homocysteine, and low or inappropriately low-normal methionine in body fluids. 5 patients became symptomatic between 5 days and 16 years and were monitored for 5 mos-7 years. [Two other infants treated pre-symptomatically are reported elsewhere.] The most common presenting symptoms were lethargy, irritability, developmental delay, mental deterioration and failure to thrive. Only 1 patient presented critically ill and acidotic. Our treatment protocol includes a low protein diet, intramuscular hydroxy-cobalamin (variable frequencies), oral betaine and supplemental methionine. It differs from other regimens in the use of methionine. Methionine deficiency is hypothesized to be detrimental to the brain, and methionine levels are kept at upper normal to two-fold normal levels. All patients had dramatic reductions in homocysteine and methylmalonic acid levels. Acute metabolic acidosis did not recur after initiation of treatment. All patients are neurologically delayed to variable degrees. Two patients have retinopathy. Two patients died, one at 6 months from neurologic causes and the other at 21 years from an intracranial bleed, with blood and CSF homocysteine levels 60 fold increased (following noncompliance with treatment). The 21 yr patient also had mesangioproliferative glomerulonephritis, not previously described in Cbl C deficiency. One boy, treated since early infancy, developed chronic pancytopenia and interstitial pulmonary edema at 6 years. The pancytopenia was exacerbated by Parvovirus infection. It is not clear whether these problems can be attributed to the primary diagnosis. One patient required gastrostomy placement because of anorexia. Despite apparently good metabolic control the outcome in our group was poor. Optimal treatment and monitoring is not defined and improved treatment is needed.

Lesch Nyhan syndrome in monozygotic twins in colombia. *p. garavito¹, j.c. prieto², n. laza¹, j.c. perez¹.* 1) Universidad del Norte, Barranquilla, Colombia; 2) Pontificia Universidad Javeriana Santa Fe de Bogota, Colombia.

We report the first case of Lesch Nyhan syndrome in two male identical twins in Colombia and the second one worldwide. Lesch Nyhan syndrome results from a complete or partial deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT). The clinical symptoms in the twins started at 6 months of age with developmental delay, spasticity, seizures and choreiform movements. Compulsive self-mutilation was evident by 26 month of age. Hyperuricemia, hyperuricosuria and HPRT deficiency was confirmed. MRI revealed cortical and subcortical atrophía in both twins. Partial exodoncia was realized. At the moment, they are receiving ambulatory treatment with anticonvulsive drugs and physical therapy. Lesch Nyhan is a very uncommon pathology in identical twins and the clinical symptoms are similar with those described in the literature.

Molecular Characterization of Phenylalanine Hydroxylase Gene in PKU Patients in Brazil. *A.X. Acosta^{1,2}, W.A. Silva Jr², T.M. Carvalho³, M.C.R. Costa², M.T. Inocencio², M.A. Zago^{1,2}*. 1) Faculdade de Medicina de Ribeirao Preto-USP; 2) Hemocentro de Ribeirao Preto, Ribeirao Preto SP, Brazil; 3) Associacao de Pais e Amigos de Excepcionais - APAE-SP.

Phenylketonuria (PKU) is highly heterogeneous at the molecular level. To date more, than 400 different mutations in the PAH gene have been described, associated with different geographical and ethnic groups. The present study includes PKU patients from the southeast of Brazil. We have investigated 230 independent mutant chromosomes for the 13 different exons, and the promoter region of the PAH gene, using DGGE, SSCP and direct sequencing. 91% of the alleles were identified, and a total of 50 different mutations were detected, with 38 mutations causing disease, and the remaining as polymorphisms. Five mutations represent 50% of the Brazilian PKU alleles; the most common were IVS10 (17,4%), R261Q (12.2%), V388M (9,1%), R252W (6,5%) and R270K (4.8%). The remaining mutations are rare. The mutational spectrum includes eight novel mutations: IVS5nt-54a/g, IVS6nt17g/t, E205A, F240S, I318T, C357G, IVS11nt17g/a and S411X. The haplotypes were studied by analysis of the intronic polymorphic sites using PCR/RFLP, and the VNTR alleles for the PAH gene. Regarding the patients completely genotyped, the genotype-phenotype correlation was determined using the predicted level of phenylalanine hydroxylase activity and the pretreatment serum level of phenylalanine. Those data confirm the heterogeneity of PKU mutations and contribute to the knowledge about distribution and frequency of PKU mutations in Latin America. Financial Support: FAPESP, FUNDHERP.

Hyperammonemia and amino acid profiles in propionic acidemia. Z.N. Al-Hassnan¹, S.A. Boyadjiev¹, V. Praphanphoj¹, A. Hamosh¹, N.E. Braverman¹, G.H. Thomas², M.T. Geraghty¹. 1) McKusick-Nathans Inst.of Genetic Medicine, Johns Hopkins University,; 2) Kennedy-Krieger Inst., Baltimore, MD.

Hyperammonemia is a common complication in patients with propionic acidemia. Propionic acid has been reported to impair N-acetyl glutamate synthesis resulting in secondary inhibition of carbamyl phosphate synthase. We reviewed the medical records of 5 patients with propionic acidemia over a 16 year period. We collected information on events where plasma amino acids and ammonium, plasma amino acids and acid-base balance or all 3 parameters were obtained simultaneously. All patients were on diets restricted in specific amino acids throughout the period. In contrast to hyperammonemia in patients with a urea cycle disorder, plasma glutamine levels were below the normal mean and there was no correlation between plasma ammonium and glutamine levels. Hyperammonemia was associated with low normal levels of citrulline, arginine and ornithine. Euammonemia was associated with low normal levels of isoleucine, methionine and threonine, while valine was below the normal range; hyperammonemia was associated with a moderate rise in all of these values. All patients showed hyperglycinemia but hyperammonemia was associated with a decline in glycine levels. Plasma glycine correlated positively with serum bicarbonate i.e. low glycine was associated with acidosis. The low levels of plasma glutamine suggest that sodium phenylbutyrate or phenylacetate be used with caution in propionic acidemia and further that glutamine levels may not be predictive of cerebral edema as in urea cycle defects.

NH4 (micromol/L)	<50 (n=60)	50-99 (n=100)	>99 (n=81)	Mean (Range)
Gln (micromol/L)	413 (158-780)	388 (149-959)	386 (118-861)	505 (337-673)
Gly (micromol/L)	1033 (181-2300)	844 (182-2450)	820 (192-1864)	205 (87-323)

A fraction of Npc1 is localized to plasma membrane rafts. *R.A. Heidenreich¹, R.P. Erickson¹, J.M. Wilson¹, S. Patel², W.S. Garver¹.* 1) Depts. of Pediatrics and Cell Biology and Anatomy, University of Arizona, Tucson, AZ; 2) VA Connecticut Healthcare System Newington, CT.

The Niemann-Pick C1 (Npc1) protein has an as yet undefined role in intracellular cholesterol homeostasis. Our investigations show that the majority of Npc1 is localized to unique intracellular vesicles with some of these vesicles also containing caveolin-1. Caveolin-1, in addition to being a marker for plasma membrane caveolae, is a cholesterol binding protein. We have previously described alterations in the expression of caveolin-1 in NPC heterozygous murine tissues and cells, suggesting a functional relationship between Npc1 and caveolin-1. Using different methods to isolate plasma membrane (Nycodenz, sucrose density gradient centrifugation and cationic colloidal silica isolation) our results indicate that a fraction (10-15%) of Npc1 is localized to the plasma membrane. Moreover, using detergent-insoluble sucrose density gradient centrifugation a similar amount (10-15%) of Npc1 was found associated with lipid rafts that cosediment with caveolin-1. These results suggest that Npc1 localized to the plasma membrane is associated with lipid rafts, possibly caveolae, and indicates the need for further investigation of the functional relationship between Npc1 and caveolae.

A mouse model of galactose-induced cataracts. *K. Huang¹, Y. Ai¹, Z. Zheng¹, A. O'Brien-Jenkins¹, C. Ning², R. Reynolds², S. Segal², D.J. Bernard³, A. Winshaw-Boris⁴, D. Stambolian¹.* 1) Dept of Genetics and Ophthalmology, University of Pennsylvania, Philadelphia, PA; 2) Division of Biochemical Development and Molecular Diseases, The Childrens Hospital of Philadelphia, Philadelphia, PA; 3) Genetic Disease Branch of the NHGRI and NIH, Bethesda MD; 4) University of California, School of Medicine, San Diego, CA.

Galactokinase (GK; EC 2.7.1.6) is the first enzyme in the metabolism of galactose. In humans, GK deficiency results in congenital cataracts due to an accumulation of galactitol within the lens. To make a galactosemic animal model, we cloned the mouse GK gene (*Glk1*) and disrupted it by gene targeting. The livers of homozygous GK-knockout (*Glk1*⁻/*Glk1*⁻) and heterozygous GK-deficient mice displayed little and half-normal GK activity, respectively. Intraperitoneal administration of ¹⁴C-galactose into *Glk1*⁻/*Glk1*⁻ mice resulted in 10-fold decreased galactose oxidation compared to normal controls. After placement on a high galactose diet, *Glk1*⁻/*Glk1*⁻ mice, as compared to wild type controls, accumulated significantly more galactose and galactitol in the kidney, lens, and liver. Surprisingly, galactose-fed, GK-deficient mice failed to develop cataracts. Hence, we introduced the human aldose reductase (*hAR*) gene driven by the α A-crystallin promoter into GK-deficient mice. In *Glk1*⁻/*Glk1*⁻, *hAR*⁺/*hAR*⁻ mice, cataract formation and lens galactitol accumulation occurred readily in suckling mice. Cataracts and elevated lens galactitol levels were found in postnatal day 1 mice, even when the mothers were maintained on a galactose-free diet. These results indicate that galactose-induced cataracts occur early in development and do not require loading mice with exogenous galactose. This raises the question of whether galactose-free diets in human pregnant females with GK-deficiency will prevent the onset of congenital cataracts. We are currently investigating the source of galactose in *Glk1*⁻/*Glk1*⁻, *hAR*⁺/*hAR*⁻ mice.

Genetic counseling and identification of carrier females in a multigenerational family with Lesch-Nyhan

syndrome. *L.A. Niewiadomski¹, T.E. Kelly¹, J.P. O'Neill²*. 1) Dept. of Pediatrics, Univ. of Virginia, Charlottesville, VA; 2) Univ. of Vermont Genetics Laboratory, Burlington, VT.

Lesch-Nyhan syndrome is an X-linked disorder caused by deficiency of the purine-salvage enzyme HPRT. Although biochemical diagnosis in males is readily accomplished by measurement of HPRT enzyme activity in blood and tissue samples, carrier detection in females can be more difficult due to possible non-random X-inactivation and selection against HPRT deficient cells in vivo. We present a four generation kindred with two affected cousins and report results of carrier testing among females in the family. Patient 1 was diagnosed prenatally by demonstration of deficient HPRT-enzyme activity in cultured amniocytes after hair root analysis identified his mother as a carrier. Patient 2 (first cousin of Patient 1) was diagnosed with Lesch-Nyhan syndrome at 8 months of age after presenting with striking involuntary movements and hyperuricemia. His mother previously had hair root analysis with normal results. Later testing determined the ability of cultured T-lymphocytes to grow in vitro in the presence of a purine analog, TG (6-thioguanine), which only allows for mutant HPRT survival. T-lymphocytes from both affected males grew in the presence of TG but not in then presence of HAT medium, which is consistent with the diagnosis of a HPRT mutation. Carrier status in females was determined through a T-lymphocyte cloning assay, which measures the HPRT mutant frequency (MF). Although HPRT mutant cells are selected against in vivo, carriers have significantly elevated MFs compared to non-carrier females. Females in the family with affected sons had MFs consistent with the heterozygous state (1.5×10^{-2} and 1.8×10^{-2}), while a third female had a normal MF (6.5×10^{-6}). Molecular analysis of the HPRT gene in Patients 1 and 2 by genomic multiplex PCR revealed a deletion of exons 1, 2, and 3. Detection of the deletion in these females is difficult given the presence of a normal gene. This family underscores the benefit of combining biochemical, cellular and molecular methods to clarify carrier status and rapidly identify potential carriers of HPRT mutations in large families.

Presymptomatic diagnosis and treatment of cblC deficiency does not prevent the development of disease-related retinopathy. *P. Kaplan, C.P. Venditti, A. Laufer, E.M. Kaye, M. Harrington, M. Miccolis, G.T. Berry.*

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The introduction of tandem MS as a tool for screening for inborn errors of metabolism has permitted the presymptomatic detection of infants with a variety of biochemical genetic diseases. However, it is not yet known whether early identification of affected infants will ameliorate complications and/or alter the natural history and progression of disease. Two unrelated Caucasian male infants with cblC deficiency were identified because of increased propionyl carnitine detected by tandem MS analysis of a newborn blood spot. The pregnancy, labor and delivery were uncomplicated in both cases. Neither infant had medical problems in the neonatal period. Evaluation in the 2nd-3rd week of life demonstrated massively elevated methylmalonic acid and homocystine and low methionine in the CSF and blood in the setting of normal newborn behavior. Brain MRI, renal ultrasounds, and dilated eye exams were normal. Treatment with betaine, methionine, IM hydroxycobalamin given at varying intervals and protein restriction was initiated with improved biochemical parameters. Both infants developed horizontal nystagmus during the first 3-4 months of life and have severe retinal pigmentary lesions which will affect vision. At 6 and 8 months respectively the infants show mild gross motor delays. Both have required gastrostomy tube placement for feeding difficulties. It appears that vigilant nutritional and biochemical monitoring have not prevented some of the complications seen in cblC disease. However, the optimal doses and frequency of administration of medications have not been established.

Alkaptonuria: New studies of an old disease. *W.J. Introne¹, M. Rausche², Y. Anikster², F. Gilbert³, W.A. Gahl².* 1) Medical Genetics Branch, NHGRI, Bethesda, MD; 2) Heritable Disorders Branch, NICHD, Bethesda, MD; 3) Cornell Medical Center, New York, NY.

A 75-year old Dominican male with alkaptonuria was admitted to the NIH Clinical Center with bilateral hip replacements, an artificial knee, aortic valve replacement, and characteristic vertebral joint abnormalities. Urinary excretion of homogentisic acid was 3.25 grams/day (normal, <0.03 grams/day). A C120W substitution was identified in exon 6 of this patients' homogentisic acid oxidase (HGO) gene, and may represent the founding mutation common to all alkaptonuria patients within the enclave of the Dominican Republic. This patient illustrates the full extent of debilitation associated with the ochronosis of alkaptonuria, one of the first inborn errors described by Sir Archibald Garrod nearly a century ago. This rare autosomal recessive disorder, due to deficiency of HGO, results in the accumulation of homogentisic acid (HA), which subsequently polymerizes and destroys connective tissue and bone. While the cartilaginous abnormalities of alkaptonuria have been known for decades, modern radiologic techniques are only now being used to evaluate this condition. We provide examples of state-of-the-art evaluation of ochronotic tissue by displaying CT scans and MRIs performed on our severely affected patient as part of an extensive natural history protocol. We are also gathering baseline data in anticipation of therapeutic drug trials with NTBC, an insecticide that inhibits the activity of 4-hydroxyphenylpyruvate dioxygenase, the enzyme in the catabolic pathway of tyrosine that produces HA. NTBC should effectively eliminate the accumulation of HA. In preparation for treatment with NTBC, we are determining which clinical signs will best serve as outcome parameters during NTBC use.

Two siblings with the congenital neuromuscular form of glycogen storage disease, type IV. *T. Jewett¹, C. Stanton², D. Jason², L. Terry¹.* 1) Department of Pediatrics, Division of Medical Genetics, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157; 2) Department of Pathology, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157.

Glycogen storage disease, type IV (GSD-IV) is the rarest of the glycogen storage diseases and is caused by a deficiency of the branching enzyme α -1, 4-glucan: α -1, 4-glucan 6-glycosyltransferase. GSD-IV is an autosomal recessive condition which varies with respect to age of onset and natural history. The most common ("classical") form presents in the first eighteen months of life and is characterized by progressive liver cirrhosis. Children with the classical form usually undergo liver transplantation or die by five years of age. In some, however, the liver disease is not progressive. In addition to the classical form, a congenital neuromuscular form has been rarely reported. Affected infants present at birth with severe hypotonia, muscle atrophy, and impairment of CNS function culminating in death in the neonatal period.

We report two siblings with the congenital neuromuscular form of GSD-IV, bringing the number of affected individuals described to a total of seven. Clinical history, autopsy findings, and biochemical test results are presented with a review of current knowledge of the glycogen branching enzyme (GBE) gene. Plans are underway for molecular analysis in this family.

Glycogen storage disease type Ib without neutropenia. *Y. Matsubara¹, S. Kure¹, Y. Suzuki¹, D.-C. Hou¹, Y. Suzuki², K. Narisawa¹.* 1) Department of Medical Genetics, Tohoku University School of Medicine, Sendai, Japan; 2) Department of Pediatrics, Gifu University School of Medicine, Gifu, Japan.

Glycogen storage disease type Ib (GSD-Ib) is an inborn error of glycogen metabolism caused by defects in microsomal transport of glucose-6-phosphate. Unlike patients with glycogen storage disease type Ia (GSD-Ia) who are deficient in the catalytic subunit of glucose-6-phosphatase (G6Pase) activity, those with GSD-Ib commonly show persistent neutropenia and impaired neutrophilic function. We have previously identified various mutations among Japanese patients with GSD-Ib, including a common W118R mutation which accounted for 50% of mutant alleles in the population (Kure et al. BBRC 248:426-431, 1998; Hou et al. AJMG 86:253-257, 1999). Here we report two atypical GSD-Ib patients without neutropenia or infectious complications. Although their clinical pictures were compatible with GSD-Ia, neither patient had abnormalities in G6Pase by enzymatic or genetic analysis. An analysis of the G6P transporter gene showed that patient 1 was a compound heterozygote of G339D and R415X mutations, while patient 2 was a homozygote of 794g>a mutation. The 794g>a mutation altered the last nucleotide of exon 3, resulting in the skipping of exon 3 which would produce premature termination at codon 130. Neutropenia-negative GSD-Ib appeared to be caused by distinct mutations that have some residual transport activities. Our results suggest a distinct clinical entity of GSD-Ib that lacks neutrophilic abnormalities. When no abnormalities is detected in the G6Pase gene, analysis of G6P transporter should be considered even if the patient does not have neutropenia or infectious complications.

Prevalence of the Y438N defect in non-Mennonite Maple Syrup Urine Disease patients: *Founder effect or de novo event?* L.D. Love-Gregory¹, R.E. Hillman², C.L. Phillips^{1,2,3}. 1) Genetics Area Program; 2) Department of Child Health, Division of Medical Genetics; 3) Department of Biochemistry, University of Missouri-Columbia, Columbia, MO.

Maple Syrup Urine Disease (MSUD) is genetically heterogeneous, resulting from mutations in any catalytic component of the branched chain α -keto acid dehydrogenase (BCKAD) complex. Different MSUD mutations are expected to arise on distinct genetic backgrounds unless there is evidence of relatedness among families or independent origins of a particular allele. For example, MSUD in Mennonites is characterized by a tyrosine to asparagine substitution (Y438N; formerly Y393N) in the E1 α subunit of BCKAD. Based on pedigree analyses, the presence of this allele in all reported Mennonite MSUD families is attributed to a *founder effect*. Hence, families that are not of Mennonite descent are not expected to carry Y438N unless it occurred as an independent event.

We have identified the Y438N defect in 8/43 non-Mennonite MSUD patients (2 homozygotes, 6 compound heterozygotes) and are currently evaluating the genetic origin of the Y438N allele in this group, by analyzing microsatellite markers that closely flank the BCKDHA gene (encodes E1 α and location of Y438N) on chromosome 19q13.1-13.2. Here we report haplotype analyses performed on four Mennonite MSUD families (37 individuals) and 6 non-Mennonite patients and available family members (16 individuals) using markers D19S223 and D19S200 (chosen from the Genome Database). Our results demonstrate a haplotype that co-segregates with the Y438N allele on chromosomes of only Mennonite MSUD patients, carrier relatives, and one Y438N compound heterozygote non-Mennonite patient and carrier parent. In addition to this major haplotype, three minor haplotypes were detected on the remaining non-Mennonite Y438N allele chromosomes. These results may represent independent origins of Y438N, alternatively they may result from age-old recombinations of the major haplotype. In the future, more markers will be used to confirm the nature of the common Y438N MSUD allele to provide further knowledge into its biological significance.

A Novel Mechanism for Dysfunction of Biotin Homeostasis. *R. Mardach*¹, *J. Zemleni*², *D. Mock*², *B. Wolf*³, *S. Cress*¹, *J. Boylan*¹, *S. Roth*¹, *S.D. Cederbaum*⁴. 1) Regional Metabolic Service, Kaiser Permanente, Los Angeles, CA; 2) Division of Gastroenterology, Hepatology and Nutrition, University of Arkansas for the Medical Sciences, Little Rock, AK; 3) Department of Human Genetics, Medical College of Virginia at Virginia Commonwealth University, Richmond, VA; 4) Division of Genetics, Depts of Psychiatry and Pediatrics, UCLA, Los Angeles, CA.

A previously healthy 22 month-old-boy, the product of non-consanguineous parents, was suspected to have a defect of biotin metabolism when a characteristic organic acid pattern in urine was found after he developed acute neurological deterioration following a viral illness. Clinical response to biotin supplementation and subsequent re-appearance of the organic acid pattern upon discontinuation of the biotin heightened the likelihood of biotin related dysfunction. However, an enzymatic deficiency was not demonstrated when blood cell biotinidase, fibroblast holocarboxylase synthase and individual carboxylase activities were measured. In addition, no mutations in the exons of the biotinidase gene were found. The patient's urinary excretion of biocytin and biotin were abnormally increased even when there was biochemical evidence of cellular biotin shortage (elevated 3-hydroxyisovaleric acid). These data were consistent with biotin wasting leading to biotin deficiency at the tissue level, and an inadequate biotin regulatory mechanism. Biotin uptake into fresh lymphocytes is severely impaired (5-10% of normal). This patient may represent the first demonstrated case of deficiency of a biotin transporter in man.

Measurement of in vivo urea cycle flux in the evaluation and management of urea cycle disorders. *B. Lee¹, W. O'Brien¹, J. Rosenberger^{2,3}, P. Reeds^{2,3}, Q. Zheng¹, F. Scaglia¹.* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 3) Children's Nutrition Research Center, Baylor Col Medicine, Houston, TX.

Diagnosing partial urea cycle deficiencies based on clinical and laboratory data is difficult since there is often poor correlation between genotype, in vitro enzyme activity, and phenotype. In the past we have evaluated patients with urea cycle disorders by using stable isotopic measurements to obtain a direct estimate of in vivo urea cycle activity. We have used the ratio of ^{15}N urea/ ^{15}N glutamine (^{15}N U/G) to distinguish asymptomatic and symptomatic ornithine transcarbamylase deficiency (OTCD) carriers, their affected offspring, and normal controls. The results suggest that glutamine labeled with ^{15}N in its amide group, provides a direct measure of urea cycle activity and can differentiate these different groups.

This method has now been used to evaluate at risk female OTCD carriers, and to compare different utilization of enteral vs. systemic sources of nitrogen for total body urea production. We determined the ^{15}N U/G ratio to evaluate the carrier status in 4 at risk females in one OTCD sibship in which mutations were not found in the male proband. Results demonstrated that in vivo measures of ureagenesis can complement other studies in the diagnosis of partial urea cycle disorders. Finally we addressed the hypothesis that intestinally generated urea precursors are more efficient precursors of urea synthesis than peripherally generated glutamine and subsequently patients with compromised urea cycle activity would metabolize excessive dietary protein more effectively than nitrogen generated peripherally. By measuring the proportional incorporation of ^{15}N from peripheral vs. enteral sources of $^{15}\text{NH}_4\text{Cl}$ and ^{15}N alanine, controls (N=12) and partial OTCD patients (N=12) were better able to convert dietary nitrogen into urea, suggesting that partial OTCD patients may be prone to hyperammonemia during stress and fasting. These data may help to guide management of these patients during episodes of catabolic stress.

Glycobiologic approaches to a novel case of Congenital Disorder of Glycosylation (CDG). *E. Orvisky¹, H. Thackray², E. Sidransky¹, M. Parker², E. St. John², D. Krasnewich¹.* 1) NIH/NIMH, Bethesda, MD; 2) NIH/NHGRI, Bethesda, MD.

CDG is a group of metabolic disorders, with a spectrum of neurologic and multisystemic manifestations caused by the defective synthesis of N-linked oligosaccharides. An 11 year boy with severe developmental delay, seizures, coagulopathy and intermittent anasarca was diagnosed with CDG based on abnormal, serum transferrin isoelectric focusing pattern. Phosphomannomutase activity was normal. In vitro studies of the synthesis of N-linked glycans in transformed lymphoblasts were performed to identify the defective enzyme. The assembly of N-linked oligosaccharides includes about 200 steps, each of which may be the defective step in different CDG types. Lymphoblasts were radiolabelled in vitro with 2-3H mannose(man) for 30 minutes. The radiolabelled man is incorporated into dolichol-linked oligosaccharides (DLO) in the earliest part of the synthetic pathway and eventually incorporated into protein-linked oligosaccharides (PLO) in the distal steps of the pathway. Lymphoblasts from this child had only 70% of normal 3H-man uptake. Radiolabelled man incorporation into both DLO and PLO was 60% and 70% of control cells, respectively. However, a substrate used in the addition of man to the growing oligosaccharides, dolichol-P-man, was found to be 160% that of normal. In order to assess the utilization of mannose-P-dolichol further, 3H-man incorporation into intermediates in the DLO synthetic pathway was performed by isolating these intermediates using an anion exchange HPLC with pulsed amperometric detection after in vitro radiolabelling. While pools of dolichol-man₈ and dolichol-man₉ in the patients lymphoblasts were 150% and 125% of control, respectively, a subsequent intermediate, dolichol-man₉glc₃, was only 70% of that found in normal cells. This suggests a buildup of precursors, dolichol-man₈ and dolichol-man₉, with a block in one of the next three steps producing dolichol-man₉glc₃, focusing the site of the possible defect. The glucosyltransferases involved in these three steps are currently being assayed and may be the underlying metabolic defect in this child with a novel type of CDG.

Clinical presentations of American patients with Congenital Disorders of Glycosylation (CDG). *D. Krasnewich*¹, *D. Andrews*², *N. Tayebi*², *O. Goker*¹, *E. Orvisky*², *E. Sidransky*¹. 1) NIH/NHGRI, Bethesda, MD; 2) NIH/NIMH, Bethesda, MD.

Congenital Disorders of Glycosylation are a relatively new class of rare metabolic diseases resulting from the abnormal synthesis of N-linked oligosaccharides. This diagnosis is made by the distinctive presence of cathodally migrating bands on serum transferrin isoelectric focusing. The clinical spectrum of CDG has expanded dramatically in the past 5 years. Many children present with severe neurologic manifestations and multisystemic involvement while others have normal cognitive development with neurologic or gastrointestinal symptoms. The developmental and neurologic course of affected children and adults, as well as the spectrum of systemic complications of these disorders continues to be delineated with each case diagnosed. Sixteen American children and adults with CDG were seen at the National Institutes of Health over the past 4 years. The ages of the patients studied ranged from 6 months to 32 years including the 3 oldest known American patients. Eleven were CDG type Ia, one was CDG type Id and four were diagnosed with CDG but no specific metabolic defect could be defined. All patients had developmental delay ranging from moderate to severe, 13/16 had seizure disorders, and 5/16 had retinitis pigmentosa. None of the patients could walk without support. In all cases, feeding difficulties, failure to thrive, and vomiting were reported, within the first year of life. During childhood, recurrent diarrhea was common (25%). Liver dysfunction was documented in 9/16 patients. Although typically high, liver transaminases returned to normal in the majority (80%) of cases within the first 2 years of life. Genotypic analysis of PMM2 performed on patients with CDG type 1a revealed a range of genotypes and, as expected, R141H was the most common mutation encountered. Studies of the natural history, genotype/phenotype correlation and clinical complications of patients with CDG will enable better assessment of prognosis and help to target effective interventions.

Paternal germline mosaicism in OTC deficiency. *R.A. Slaugh¹, D.K. Grange², M. Tuchman³, R.A. Martin¹.* 1) Peds/Div Medical Genetics, Washington Univ, St Louis, MO; 2) Peds/ St. Louis Univ, St Louis, MO; 3) Children's National Medical Center, Washington, D.C.

Ornithine transcarbamylase (OTC) deficiency is an X-linked disorder of the urea cycle. The classic presentation in males is severe hyperammonemia in the newborn period while "milder" mutations can present at any age. The presentation in heterozygous females varies from asymptomatic to severe. OTC deficiency is usually maternally inherited or is the consequence of a new mutation, but parental germline mosaicism has been reported in one mother who had two affected sons and in one father who had three presumably affected daughters. We present two paternal half-sisters with OTC deficiency. Both are heterozygous for the same previously unreported mutation in the OTC gene.

The proband presented at 17 months of age with a three day history of lethargy and irritability. OTC deficiency was diagnosed. DNA analysis demonstrated a TTT to TCT point mutation in codon 316 of exon 9 resulting in the amino acid change Phe-316-Ser. This mutation is believed to be deleterious although it had not previously been described. The mutation affects a phenylalanine residue that is located at the interface between the polypeptides interacting with the conserved aspartate 126 of the adjacent monomer. DNA analysis of peripheral blood leukocytes in the proband's mother did not identify this mutation. One year later, the proband's paternal half sister presented at 6 months of age with hyperammonemic coma, a stroke-like lesion of the right cerebral hemisphere and cerebral edema. Mutation analysis revealed the same mutation as was found in the proband.

The father of both girls is unavailable for clinical evaluation or DNA studies. It is presumed that he has germline mosaicism for this OTC mutation. Thus, the father of a girl with OTC deficiency may have germline mosaicism for a mutation in the OTC gene and may be at risk to have multiple affected daughters. Parental germline mosaicism should be considered when evaluating and counseling a family regarding the risk to have additional affected children.

Regulation of Human Galactose-1-Phosphate Uridyltransferase (hGALT) Gene Expression. *C. Saunders, S. Langley, K. Lai, L.J. Elsas.* Division of Medical Genetics, Department of Pediatrics, Emory University, Atlanta, GA.

Two biochemical phenotypes for galactose-1-phosphate uridyltransferase (hGALT) have been defined for the same molecular genotype in patients with the hGALT missense mutation, N314D. In lymphoblasts transformed from homozygotes (N314D/N314D), the Duarte (D₂) variant has 50% GALT whereas the LA (D₁) variant has normal GALT activity and a linked polymorphism, 1721C®T. We previously found decreased thermostability in purified hGALT with N314D and decreased mRNA and biostability in homozygous lymphoblasts (N314D/N314D). Here we compare and evaluate gene expression of Duarte (D₂) and LA (D₁) variant hGALT. We cloned and sequenced a 5' genomic fragment spanning ~4kb upstream of the transcription start site. A BLAST analysis revealed at least 16 candidate *cis*-regulatory domains in the first -698 bp. From progressive deletions we identified both positive and negative regulatory regions. A region from -165 to -101 was absolutely required for promoter activity and contained a carbohydrate response element (ChoRE) with 3 GTCA repeats. Deleting a single GTCA repeat from -119 to -116 resulted in a loss of 22±3% of luciferase activity in transfected HEK 293 cells compared to the full length promoter construct. We examined DNA from 35 patients with Duarte (D₂) and LA (D₁) alleles for this GTCA sequence and found a loss of this site in all Duarte alleles examined but in no LA alleles. Furthermore, the DGTCA segregated with the Duarte allele but not the LA allele in an informative pedigree. We conclude that the ChoRE is a positive regulatory element for hGALT, and that deleting a GTCA repeat contained within the ChoRE reduces hGALT promoter activity. Finally, the DGTCA is linked in *cis* to the Duarte (D₂) allele but is not associated with the LA (D₁) allele.

Identification of a novel mutation in patient with carnitine palmitoyltransferase II (CPT II) deficiency. B-Z.

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Carnitine palmitoyltransferase II deficiency (CPT II) is an autosomal recessive disorder of mitochondrial fatty-acid oxidation, which presents as three distinct clinical forms: an adult form, neonatal (hepatocardiomyopathy) form and a milder infantile form. In this study, a patient with CPT II deficiency was investigated. The proband presented at age 12 months characterized by hypoketotic hypoglycemia, dicarboxylic acidemia, and low level of carnitine. In vitro probe analysis with [16-²H₃] palmitic acid from the proband's fibroblasts resulted in labeled acylcarnitines consistent with either CPT II or carnitine-acylcarnitine translocase (CACT) deficiency. CPT II activity in fibroblasts was measured (0.125 nmol/min per mg protein) and found to be only 8% of the normal control indicating CPT II deficiency. To investigate the molecular basis of CPT II deficiency, all of CPT II exons from patients DNA were amplified and directly sequenced. A novel mutation was identified which was a G733T transversion in exon 4 resulting in a Valine-to-Phenylalanine substitution at residue 245 of the protein (V245P). A method based on PCR/Xcm I Restriction-endonuclease digestion was developed for the detection of the V245P mutation. This mutation was also identified in proband's affected sibling, but was not detected in the normal control subjects. In addition, one base alteration G1102A (V368I) was identified and confirmed as a polymorphism. The correlation of genotype to phenotype is briefly discussed in relation to our total 19 patients with CPT II deficiency.

Relationship between clinical phenotype, residual OTC activity and pattern of liver X inactivation in symptomatic OTC-deficient females. *F. REYAL¹, N. GIGAREL¹, D. RABIER², B. GILBERT¹, J. BARDET², L. COLLEAUX¹, JM. SAUDUBRAY³, J. AMIEL¹, A. MUNNICH¹, JP. BONNEFONT².* 1) Genetics; 2) Genetic Biochemistry; 3) Pediatrics Departments, Hopital Necker, Paris, France.

Ornithine carbamoyltransferase (OTC) deficiency is the most common inherited disease of urea cycle in human. This X-linked disease (Xp21) is almost fully penetrant in the neonatal period. Conversely, the symptoms in carrier females vary in both age of onset and severity. This variability is believed to be accounted for X inactivation skewing in liver but very few data supporting this hypothesis are available. We have analyzed the pattern of X inactivation and the residual OTC activity in liver samples from seven OTC-deficient female children with various degrees of clinical severity. Residual OTC activities ranged from 0 to 50 % while the pattern of X inactivation varied from random to a 100 % skewed X inactivation. A strong correlation between the two parameters was obvious in 6/7 patients. While a clear correlation between the clinical severity, the residual OTC activity and the pattern of X inactivation was found in the liver of two OTC-deficient female siblings, interfamilial relationship between clinical severity and biological parameters was poor in our cohort, suggesting the possible involvement of additional factors, particularly the nature of the mutant genotype. Among the three mutations identified, the nonsense substitutions (L43P and W332R) resulted in a severe neonatal disease while the splicing mutation IVS7+3A>G was associated with a "mild" phenotype. Moreover studying multiple samples (52) in one patient indicated a marked heterogeneity in both OTC activity (1 to 35 %) and pattern of X inactivation (15/85 % to 45/55 %) across various regions of the liver. Thus, the results obtained from liver biopsy are of poor value for predicting the clinical severity of OTC deficiency in females.

Mutations in the human genes encoding the MCCA and MCCB subunits of the 3-methylcrotonyl-CoA carboxylase in methylcrotonylglycinuria patients. *S. Rodriguez de Cordoba¹, M.E. Gallardo¹, J. Esparza¹, J.M. Rodriguez¹, L.R. Desviat², C. Perez-Cerda², B. Perez², P. Rodriguez-Pombo², R. Navarrete², A. Ribes³, T.P. Le⁴, M. Gibson⁵, M.A. Penalva¹, M. Ugarte².* 1) Centro Invest. Biologicas (CSIC) and Fundacion Jimenez Diaz, Madrid, Spain; 2) Centro Biol. Mol.-SO, UAM, Madrid, Spain; 3) IBC, Barcelona, Spain; 4) Dept. Pediatrics, UCSD, USA; 5) OHSU, Oregon, USA.

Methylcrotonylglycinuria (OMIM, 210200) is an autosomal recessive inherited human disorder of the leucine catabolism and isoprenoid metabolism caused by the absence of the biotin enzyme 3-methylcrotonyl-CoA carboxylase (MCC, EC 6.4.1.4). MCC-deficient patients excrete large quantities of 3-methylcrotonylglycine and 3-hydroxyisovaleric acid in the urine and many of them present muscular hypotonia and atrophy. MCC is a heteromeric enzyme composed of biotin-containing (MCCA) and non-biotin-containing (MCCB) subunits. We have isolated the full length cDNAs encoding the human MCCA and MCCB subunits and determined the structure of the human *MCCA* and *MCCB* genes. The human MCCA and MCCB polypeptides are encoded by single genes located in chromosomes 3p11.2-p13 and 5q12-q13.2, respectively. Upon transcription *MCCA* originates a transcript of 2.6 kb, whereas *MCCB* produces two transcripts of 4 and 2.4 kb that differ in their 3 untranslated regions. *MCCA* and *MCCB* encode polypeptides of 725 and 563 amino acids respectively. *MCCA* and *MCCB* expression was detected in all human tissues analyzed but it was significantly increased in heart, skeletal muscle, kidney and liver. RT-PCR and genomic sequencing of *MCCA* and *MCCB* in MCC-deficient patients of the MCCA and MCCB complementation groups has resulted in the identification of missense and frameshift mutations in *MCCA* and *MCCB*. Functional characterization of the polypeptides encoded by the *MCCA* and *MCCB* genes is in progress.

Geographic and ethnic distribution of mutations in the FAH gene of hereditary tyrosinemia type 1 patients in Europe. *J.K. Ploos van Amstel¹, R.A. Zewald¹, I.E.T. Van den Berg², R. Berger².* 1) Department of Medical Genetics, University Medical Center Utrecht, The Netherlands; 2) Laboratory for Metabolic Diseases, University Medical Center Utrecht, The Netherlands.

Hereditary tyrosinemia type 1 (HT1, MIM 276700) is an autosomal recessive disorder caused by a deficiency of the enzyme fumarylacetoacetase (FAH). Since 1993, we are involved in the molecular diagnostics of HT1. To date, more than 60 HT1 families have been diagnosed in our laboratory. The families originate from different parts of Europe and from the Mediterranean countries. Of all families, the two deleterious FAH gene defects have been identified. To summarize, 34 different mutations have been found viz. deletions (3), insertions (1), nonsense mutations (4), missense mutations (17), splice mutations (9). Several mutations showed a clear ethnic/geographic origin. The IVS12+5(g-a) defect is predominant (30%) in the countries of northwest Europe, whereas the IVS6-1(g-t) splicing defect is most frequently present (55%) in the mediterranean countries. All three patients from Balkan countries were compound heterozygous for a Met1Val missense mutation. Patients from jewish origin were homozygous for a P261L missense mutation. Two patients showed beside two HT1 mutations also heterozygosity for R341W the frequent pseudodeficiency mutation. Furthermore, more than half of the probands showed homozygosity. This is probably due to the relatively high frequency of the IVS mutations, and to the high rate of consanguinity of the parents. To conclude, HT1 is molecular heterogeneous, albeit that there exist a significant geographic and ethnic distribution of several mutations. This enables the development of a strategy in the genetic testing that can be based on origin of the proband and frequency of the mutations before the complete gene is sequenced.

Transient dicarboxylic aminoaciduria associated with poor weight gain in a newborn. *D.J. Waggoner¹, J. Keating², M. Landt².* 1) Dept Human Genetics, The Univ of Chicago, Chicago, IL; 2) Dept Pediatrics, Washington Univ, St. Louis, MO.

The existence of a common renal transport mechanism for glutamate and aspartate has been known for some time and dicarboxylic aminoaciduria has been reported but is relatively uncommon. These biochemical findings have been associated with a variable phenotype ranging from normal adults to growth failure and mental retardation with a propensity for hypoglycemia. One of the glutamate/aspartate transporter genes, EAAC1, is expressed in brain, small intestine and kidney and is a potential candidate responsible for the cases of dicarboxylic aminoaciduria with mental retardation. The finding of individuals with no phenotype however, suggests that there is no relationship between these amino acid findings and clinical expression. We report on a 2 month old female evaluated for poor weight gain and developmental concerns whose initial evaluation revealed isolated dicarboxylic aminoaciduria with a 5-fold elevation in urinary aspartate and a 30-fold elevation in urinary glutamate. One week later repeat urinary amino acids showed decreased dicarboxylic aminoaciduria with 2.5-fold elevation in aspartate and 20-fold elevation in glutamate. Approximately 1.5 months after the initial evaluation her urine amino acid levels had returned to normal. Concomitantly with the resolution of her laboratory abnormalities, her energy levels, feeding and weight gain improved to normal. These observations suggest that dicarboxylic aminoaciduria and failure to thrive may be related and that the expression of glutamate transporters may be developmentally regulated. Further observations of a similar phenomenon may help clarify the expression patterns of glutamate transporters in children and further define their association with neurological findings.

Mutation spectrum and haplotype analysis of holocarboxylase synthetase gene. *Y. Suzuki¹, X. Li¹, X. Yang¹, O. Sakamoto¹, Y. Aoki¹, M. Hiratsuka¹, K. Inui², M. Gibson³, T. Suormala⁴, R. Baumgartner⁴, S. Kure¹, Y. Matsubara¹, K. Narisawa¹.* 1) Dept Medical Genetics, Tohoku Univ Sch Medicine, Sendai, Japan; 2) Dept Pediatrics, Osaka Univ Sch Medicine, Osaka, Japan; 3) Dept Molecular and Medical Genetics, Oregon Health Sciences Univ, Portland, OR; 4) Metabolic Unit, Univ Children's Hospital, Basel, Switzerland.

Holocarboxylase synthetase (HCS) catalyses biotin incorporation into four carboxylases. Since biotin is essential for their enzymatic activities, defects in HCS result in decreased activity of multiple carboxylases, affecting various metabolic pathways. HCS deficiency (McKusick 253270) is therefore called also as biotin-responsive multiple carboxylase deficiency. We have identified 24 mutations in 11 Japanese and 10 non-Japanese families with HCS deficiency. The results showed that: 1) there was no common mutation; 2) two mutations (508Arg>Trp and 550Val>Met) were found in multiple ethnic groups; 3) none of three mutations (942insA, 237Leu>Pro, and 1067delG) found in multiple Japanese families was found in non-Japanese patients. These findings suggest that the spectrum of mutations in the HCS gene is different among different ethnic groups. We also determined haplotypes of the HCS gene in Japanese patients and control subjects by typing two tetranucleotide repeat markers in the gene. Fourteen haplotypes were found in 100 alleles of the control subjects. All seven 237Leu>Pro mutant alleles and all four 1067delG mutant alleles were associated with 2-2 haplotype. Both 508Arg>Trp and 550Val>Met mutant alleles were associated with either 2-3 or 1-4. The data suggest that the 237Leu>Pro and 1067delG show a founder effect in Japanese population, whereas 508Arg>Trp and 550Val>Met represent hot spot mutations.

Hypertension and adrenal insufficiency in a patient with Smith-Lemli-Opitz syndrome. *V.M. Siu¹, M.J.M.*

Nowaczyk². 1) Medical Genetics Program of Southwestern Ontario, Dept of Pediatrics, University of Western Ontario, London, ON; 2) Dept Pathology and Molecular Medicine, and Pediatrics, McMaster University, Hamilton, ON, Canada.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive MCA-MR disorder caused by mutations within the 7 dehydrocholesterol reductase (7DHCR) gene. The diagnosis is based on the biochemical findings of elevated plasma 7-dehydrocholesterol (7DHC) levels and low cholesterol levels. Adrenal insufficiency with hyponatremia has been reported in 3 previous patients with SLOS, presumed to be due to aldosterone deficiency and responding to mineralocorticoid replacement. Hypertension is not a recognized complication of SLOS. The proband was born at 36 weeks gestation weighing 1930 g to nonconsanguineous parents. Features included hypotonia, microcephaly, short anteverted nares, micrognathia, cleft palate, wide-spaced nipples, hypoplastic thumbs, bilateral cryptorchidism, microphallus, penoscrotal hypospadias, 2-3 syndactyly of the toes, and Hirschsprung disease. By day 4, he was hyponatremic and responded to sodium supplementation, then developed hypertension which persisted throughout life, despite treatment with antihypertensives, including beta blockers, vasodilators, and ACE inhibitors. Renal ultrasound showed minimal fullness of the pelvicalyceal system. Urine sodium was markedly increased with low urine osmolality and returned to normal levels after corticosteroids and mineralocorticoids were introduced on day 24. Renal function remained normal. Serum testosterone levels were normal but did not respond to hCG stimulation. Testosterone to DHT ratio was normal. Direct hyperbilirubinemia and liver transaminases persisted after treatment with ursodiol was instituted. Cholesterol treatment was started on day 26. By two months of age, he had developed biventricular hypertrophy. Despite multiple interventions, the baby failed to thrive and showed no developmental progress, dying at 3 months. We postulated that the hypertension in this infant may have been related to activation of the angiotensin system by the initial hyponatremia, but treatment with an ACE inhibitor was unsuccessful.

Aberrant splicing by a point mutation within exon 5 of the glucose-6-phosphatase gene is the major mutation identified in Korean patients with glycogen storage disease type Ia. *G.H. Kim¹, J.W. Cheong², E.J. Seo¹, H.W. Yoo¹.*

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Glycogen storage disease (GSD) type Ia (von Gierke disease) is an autosomal recessively inherited metabolic disorder caused by a deficiency in microsomal glucose-6-phosphatase(G6Pase). G6Pase catalyzes the terminal step in gluconeogenesis and glycogenolysis. We have analyzed the G6Pase gene of 15 unrelated Korean patients with GSD type Ia using genomic DNA isolated from peripheral leukocytes or cDNA from hepatocytes. PCR-SSCP analysis or heteroduplex assay with subsequent direct sequencing strategies were utilized. We identified 7 different base changes; R83H, G122D, G to T at nt. 727, C deletion at nt. 961, T202A, S284Y, and T to C at nt. 1176. Among these, T202A, S284Y, and T to C at nt. 1176 are polymorphisms. The G to T transversion at nt. 727 in coding region of exon 5 resulted in aberrant splicing at RNA processing, generating 91 base pair deletion from 5' site of exon 5. This mutation has been originally described in Japanese patients with GSD type Ia, and known to be the most common mutation in Japanese patients. In this study, we found this mutation in 17 out of 30 alleles(57% of allele frequency) from 15 unrelated Korean patients with GSD type Ia. Other mutations(R83H, G122D, C deletion at nt. 961) were found in three patients; a homozygote of C deletion at nt. 961 or two compound heterozygotes of R83H/ G to T at nt. 727 and G122D/ G to T at nt. 727. It is interesting that one major mutation is shared in both Korean and Japanese patients with GSD type Ia . However, the allele frequency of this major mutation is higher in Japanese(~90%) than in Korean patients(57%) with GSD type Ia. It indicates this mutation might have been introduced by a founder effect in both ethnic groups. In conclusion, the genotype of the G6Pase gene in Korean patients with GSD type Ia is genetically homogeneous. DNA molecular diagnostics can be utilized for the diagnosis of this disease prenatally and postnatally.

Near-syncopal episodes in an adult with mild type Hunter Syndrome. *H. Thackray, I. Abdulla, D. Krasnewich.*
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Hunter syndrome (mucopolysaccharidosis II) is an X-linked recessive lysosomal storage disease with a range of phenotypes. The metabolic defect, iduronate-2-sulphatase deficiency, leads to accumulation of dermatan sulfate and heparan sulfate in tissues, causing a recognizable clinical course. In the mild form, patients have no mental retardation and can survive to middle age. However, there is relatively little known about the natural history of this disease in adulthood. We report a 37-year old male with mild Hunter syndrome who presents with symptoms within the known clinical spectrum, including normal intelligence, coarse facial features, hoarse voice, short stature, premature degenerative disk disease, hearing loss, joint contractures, obstructive pulmonary disease, and mitral valve stenosis. He had adult-onset near-syncopal episodes not previously described in the literature. Workup for this syncope included cardiac evaluation with EKG, ECHO, and holter monitor, chest x-ray, spine MRI, pulmonary function tests, and blood tests. While findings of the above tests revealed degenerative disk disease, obstructive pulmonary disease, and mitral valve stenosis, a potential cause of the described episodes was not uncovered. On brain MRI, our patient had changes reflecting the accumulation of mucopolysaccharide throughout the brain, prominently in the basal ganglia and periventricular regions. There were also signs of chronic ischemia in the posterior limbs of the internal capsules, periventricular regions, and brain stem. MRA showed tapering of vessels in the Circle of Willis consistent with external compression of the arteries. Brain MRI findings reported in the literature include changes caused by mucopolysaccharide deposits. However, to our knowledge there is no reported association of these findings with clinical sequelae. We believe a possible explanation for the near-syncopal episodes in our patient is the compression of cerebral arteries seen on MRA. The association of near-syncopal episodes with cerebral vascular findings is previously unreported in the Hunters syndrome literature. Both the MRI and MRA findings and the new clinical features discussed elucidate the natural course of Hunters syndrome.

Evaluation of elevated hydroxyisovalerylcarnitine in the newborn screen by tandem mass spectrometry. *W.E. Smith¹, J. Muenzer², D. Frazier², D.S. Millington¹, P.S. Kishnani¹, M. McDonald¹, D.D. Koeberl¹.* 1) Dept. of Pediatrics, Div. of Medical Genetics, Duke University Medical Center, Durham, NC; 2) Dept. of Pediatrics, Div. Of Genetics, University of North Carolina, Chapel Hill, North Carolina.

Tandem Mass Spectrometry (TMS) is a powerful technique for the detection of metabolic disorders. Although very sensitive, TMS is not entirely specific and the detection of abnormal but non-diagnostic metabolites is common. We evaluated the relevance of elevated 3-hydroxyisovalerylcarnitine (C5-OH) in TMS newborn screening. Since the addition of TMS to the North Carolina Newborn Screen in April 1999, six asymptomatic infants have had two abnormal levels of C5-OH on their whole blood spot acylcarnitine profile. A plasma acylcarnitine profile and urine organic acids were obtained on all six infants: five infants had variable amounts of 3-hydroxyisovaleric acid in their urine and three of these infants had increased plasma levels of C5-OH. The latter three infants had additional elevations of both 3-hydroxyisovaleric acid and 3-methylcrotonylglycine in their urine, consistent with 3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency. Urine organic acid analysis of the mothers of two of these patients was normal. One infant has been diagnosed with 3-MCC deficiency; her enzyme activity was 43% of control activity in lymphocytes and 2% of control activity in fibroblasts (UCSD Biochemical Genetics Laboratory). Therefore, of these six infants with elevated C5-OH on TMS newborn screening, one has been diagnosed with 3-MCC deficiency and two have been identified with probable 3-MCC deficiency. Additional infants with persistently elevated levels of C5-OH on TMS newborn screening are being evaluated. Absolute levels of C5-OH on newborn screening samples were not predictive of a final diagnosis. Evaluation of newborns with persistently elevated C5-OH levels should include, at least, urine organic acid analysis and a plasma acylcarnitine profile. Long-term follow-up is needed to determine if early diagnosis and treatment of 3-MCC deficiency will allow sustained normal growth and development.

Primary hyperoxaluria in the Canary Islands: founder effect for the Ile244Thr mutation. *E.C. Salido^{1,2}, A. Santana², A. Alvarez², V. Lorenzo², A. Torres², L.J. Shapiro¹.* 1) Dept Pediatrics, Univ California, San Francisco, CA; 2) Unidad Investigacion, Hospital Universitario Canarias, Spain.

Primary hyperoxaluria type I (PH1) is a rare autosomal recessive disorder caused by mutations in the alanine:glyoxylate aminotransferase gene (AGXT). We studied 15 PH1 patients with end-stage chronic renal failure, belonging to 12 families independently ascertained over the last decade. They included one marriage between first cousins. SSCP was performed to screen for mutations at AGXT after amplification of its 11 exons. Direct DNA sequencing of exons showing aberrant migration patterns revealed various polymorphisms and a highly prevalent mutation. Of the 24 PH1 chromosomes from the Canary Islands, 22 (91.6%) carry an Ile244Thr mutation, with 13 out of 15 patients being homozygous for it. This mutation had been previously described mostly in compound heterozygous patients, accounting for 9% of the PH1 alleles. Four polymorphisms within AGXT (Pro11Leu, intron 1 ins, C386T, Ile340Met) were also typed in our patients and their relatives, and a shared haplotype (Leu-ins-T-Met, respectively) was present in all the chromosomes with the Ile244Thr mutation. Thus, the high prevalence of this mutation in the Canary Islands is most likely the result of founder effect. Ile244Thr has been accepted to be a disease causing mutation based on phenotype-genotype correlations. However, direct biochemical evidence about its functional consequences. We performed site directed mutagenesis to reproduce the mutation in cloned AGXT cDNA. HeLa cells, which do not express AGXT, were transfected with either wild type or Ile 244Thr constructs. No differences were found in AGXT enzymatic activity in cell extracts from either expression. Also, Western blot analysis revealed no differences in either abundance or SDS-PAGE migration. However, the Ile244Thr change might affect postranslational processes. For instance, Gly170Arg, the most frequent AGXT mutation, affects the subcellular localization of a catalytically active enzyme. Gly170Arg, in concert with the Pro11Leu polymorphism, delays AGXT dimerization and allows mistargeting to the mitochondria. We are studying whether Ile244Thr operates similarly.

Clinical and molecular characteristics in Korean patients with propionic acidemia. *K.H Ryu, L.H. Lee, S.H. Hahn.*
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Propionic acidemia (PA) is an autosomal recessive inborn error of the catabolism of methionine, isoleucine, threonine, valine, odd-numbered chain length fatty acids, and cholesterol. Clinical symptoms are very heterogeneous characterized by metabolic acidosis, vomiting, lethargy and hypotonia. It is caused by a deficiency of propionyl CoA carboxylase (PCC), a biotin dependent enzyme that catalyzes the carboxylation of propionyl CoA to D-methylmalonyl CoA. PCC is a heteropolymeric enzyme composed of α and β subunits, encoded by the PCCA and PCCB genes, respectively. We identified 5 Korean patients with PA by organic acid analysis confirmed by the PCC enzyme assay in the lymphoblasts. Biotinidase and methylcrotonyl CoA carboxylase were in the normal range excluding either biotin metabolism or holocarboxylase defect. Two of them who experienced their first episode of illnesses in a week after birth had undetectable PCC activities while the rests had relatively late manifestations during the infancy with developmental delay or seizure with their enzyme activities between 1.6% and 9.6% of normal. One of the patients with developmental delay did not experience any severe ketoacidosis during the life implying that PA could present as a pure neurologic dysfunction regardless of frequent episodes of metabolic ketoacidosis. In molecular analysis, mRNA slot blots showed the normal amount of mRNA of both α and β subunit genes. Since the α subunit is more unstable, the β subunit gene defect was suspected. By the RT-PCR and amplification of genomic DNA of PCCB gene, we identified two novel mutations, Y439C and 1527-1529delTTC and one known mutation, T428I. Allele frequency of T428I was 37.5% in this study suggesting that Korean patients with PA are frequently caused by one common mutation and other heterogeneous mutant alleles in PCCB gene. In conclusion, null enzyme activity of PCC appears to be related to earlier onset, however, neurologic dysfunction could occur regardless of previous ketoacidotic episodes. PCCB gene defect is responsible for the majority of Korean patients with PA and T428I appears to be the most frequent causative mutant allele.

An alternate form of Ku is present in mammalian mitochondria. *G.P. Coffey, C. Campbell.* Department of Pharmacology, University of Minnesota Medical School, Minneapolis, MN.

We have recently shown that mammalian mitochondrial protein extracts possess a DNA end-binding (DEB) activity that closely resembles Ku. Ku is a heterodimeric nuclear protein of 70 and 80 kDa subunits termed Ku70 and Ku80, respectively, and is involved in DNA double-strand break repair. This observation led us to hypothesize that mitochondrial DEB activity is dependent upon alternate forms of Ku70 and Ku80 that are targeted to the mitochondria. As an initial test of this hypothesis, we assayed for Ku activity in mitochondrial extracts prepared from the xrs-5 hamster cell line that lacks functional Ku80. Mitochondrial protein extracts prepared from this cell line lacked the DEB activity found in similar extracts prepared from wild-type V79 cells. Azacytidine-reverted xrs-5 cells that acquired nuclear DEB activity also acquired mitochondrial DEB activity. Western blot analysis of human mitochondrial protein extracts using a monoclonal antibody specific for Ku70 detected a 70 kDa protein in mitochondrial extracts. Further, a monoclonal antibody specific for an N-terminal epitope of Ku80 identified a mitochondrial protein with an apparent molecular weight of 68 kDa. This mitochondrial protein was not detected by a monoclonal antibody specific for an epitope at the C-terminal end of Ku80. Consistently, while both the N- and C-terminal Ku80 monoclonal antibodies supershifted the nuclear DEB complex on an electrophoretic mobility shift assay, only the N-terminal monoclonal antibody supershifted the mitochondrial DEB complex. Further, immunocytochemical analysis revealed the co-localization of the N-terminal specific Ku80 monoclonal antibody with a mitochondrial-targeted green fluorescence protein. Mitochondrial localization of the C-terminal Ku80 monoclonal antibody was not observed. These data are consistent with the hypothesis that a C-terminally truncated form of Ku80 is localized in mammalian mitochondria where it functions in a DEB activity. Our laboratory is currently undertaking studies to investigate the role of this DEB activity in mitochondrial DNA maintenance.

The mitochondrial DNA depletion syndrome may present with clinical, histologic, and electromyographic features of spinal muscular atrophy. *G.M. Enns¹, T.H. Vu², S. Schelley¹, C.L. Hoppel³, D. Horoupian¹, S. Shanske², S. DiMauro².* 1) Depts Ped & Pathol, Stanford Univ, CA; 2) Dept Neurol, Columbia Univ, College of Physicians & Surgeons, NY; 3) Depts Med & Pharmacol, CWRU, OH.

The mitochondrial DNA (mtDNA) depletion syndrome is an autosomal recessive, heterogeneous condition characterized by a decrease in the amount of mtDNA. Patients commonly present with myopathy or hepatopathy. Muscle biopsy typically shows ragged-red fibers (RRF) and cytochrome c oxidase (COX) deficiency, with variable other respiratory complex deficiencies. In order to delineate further the clinical spectrum associated with the mtDNA depletion syndrome, we report an 18-month-old boy who presented with features of spinal muscular atrophy (SMA). He was born at term to a consanguineous couple following a twin gestation. Asymmetric fetal movement was present, with his twin sister being more active. Polycythemia, mild hyperbilirubinemia, and mild hypotonia were present at birth. Hypotonia, absent upper extremity reflexes, and diminished lower extremity reflexes were noted at 3 months. The CK level was 3,434 IU/L and a head MRI was normal. At 7 months, electromyographic studies were consistent with anterior horn cell disease, but deletion analysis of the SMN gene was negative. A muscle biopsy showed changes suggestive of a severe neurogenic process. There were some COX-negative fibers, although none was RRF, and electron microscopy showed normal mitochondria. Muscle biochemistry showed normal complex I-IV activities, when referred to a low citrate synthase activity. Quantitative mtDNA analysis detected a 73% decreased amount of mtDNA compared to controls (n=5). We conclude that mtDNA depletion syndrome may present with features of SMA and patients with negative SMN deletion analysis should be investigated for the mtDNA depletion syndrome. Marked elevation of CK is unusual in mitochondrial disease, but common in the mtDNA depletion syndrome, and may serve as a clue to diagnosis. Defects in enzymes needed for mtDNA replication, such as DNA polymerase gamma and mitochondrial transcription factor A, have been suggested in some cases, but the molecular basis of mtDNA depletion remains obscure.

Cardiomyopathy associated with mitochondrial biogenesis defects. *M.J. Goldenthal¹, J. Marin-Garcia¹, H.B. Sarnat².* 1) Molecular Cardiology Institute, Highland Park, NJ; 2) Dept. of Pediatrics, U. Washington School of Medicine, Seattle, WA.

Mitochondrial bioenergetic dysfunction has been frequently reported in cases of cardiac disease. Our laboratory has examined specific mitochondrial enzyme and DNA changes in a group of 42 patients with cardiomyopathy (under 21 years old) and found: (1) Cardiac respiratory enzyme dysfunction is present in over 85% of our patients as gauged by reduced level(s) of at least one respiratory complex activity. (2) Pathogenic point mutations in cardiac mtDNA can be found albeit infrequently. Pathogenic mtDNA mutations previously reported in association with cardiomyopathy were not present. One patient with dilated cardiomyopathy (DCM) harbored a novel heteroplasmic mutation (at nt 10424) altering a highly conserved nucleotide in tRNA^{Arg}. (3) Specific low-level large-scale deletions (e.g., 7.4 kb deletion) in cardiac mtDNA detectable using PCR amplification were significantly increased in incidence and abundance in patients with DCM. In addition, 2 patients with DCM have highly abundant levels of multiple mtDNA deletions in other tissues (e.g., blood and skeletal muscle) detectable both by PCR and Southern blot analysis. (4) Significantly decreased levels of cardiac mtDNA and mitochondrial respiratory complex activities were found in 2 patients with hypertrophic cardiomyopathy and in 2 patients with DCM.

In conclusion, specific pathogenic point mutations in mtDNA appear to be infrequently involved in the mitochondrial bioenergetic dysfunction detected in cardiomyopathy. Defects in the level, integrity, repair and replication of mtDNA (resulting in the generation of mtDNA deletions and/or mtDNA depletion) affecting mitochondrial function and biogenesis may be due either to nuclear DNA-encoded mutations or may arise in cardiomyopathy as a function of somatic mutation.

A novel mutation in the mitochondrial DNA tRNA aspartic acid gene (A7559G) associated with a MERRF- like syndrome. *P. Chronopoulou¹, K. Tanji¹, C. Karadimas¹, S. Shanske¹, S. Krishna¹, E. Bonilla¹, L. Rutledge², S.J. Oh³, S. DiMauro¹.* 1) Dept Neurology, College of Physicians & Surgeons of Columbia University, New York, NY; 2) Dept Neurology, University of Alabama, Birmingham, AL; 3) Dept Pediatrics, University of Alabama, Birmingham, AL.

We report a woman who presented, starting at age 10, with parasthesias, exercise intolerance, and ataxia. These gradually worsened and, in her forties, she also developed bilateral ptosis, ophthalmoplegia, and myoclonus. The patient died at age 57. Neuropathologic examination showed marked neuronal loss and gliosis in the dentate nuclei and inferior olivary complexes, mild to moderate loss of cerebellar Purkinje cells, and mild loss of melanoneurons in the substantia nigra (all of which are seen in cases of MERRF, myoclonic epilepsy with ragged red fibers). Muscle tissue taken at autopsy showed numerous cytochrome c oxidase-negative ragged red fibers (another feature of MERRF). Analysis of respiratory chain enzymes in muscle homogenate showed marked reduction of complex I, III and IV activities. Direct sequencing of all 22 mitochondrial tRNA genes revealed a novel A7559G transition in tRNA Asp. PCR/RFLP analysis showed that this nt change was heteroplasmic in the patient's muscle and blood (mutant load approx 96%). The patient had two brothers who had similar clinical pictures and died in their 60s; none of their tissues were available for study. The probanda's only child, a daughter, is asymptomatic at age 27. Her muscle biopsy showed essentially normal morphology and normal respiratory chain enzyme activities, but mutant load is 93% in muscle. We believe this mutation to be pathogenic because it has not been reported before, we did not detect it in 100 controls, and it is predicted to destabilize the tertiary structure of tRNA Asp and the capability for aminoacylation. In this family, prognosis and genetic counseling for the asymptomatic daughter are problematic. To establish the threshold for pathology associated with this mutation, we will study mutation loads in asymptomatic maternal relatives past age 50. A7559G should be added to the growing list of mitochondrial mutations in patients with MERRF-like phenotypes.

Brain gene expression analysis using DNA microarrays of SOD2 knockout mice treated with the synthetic antioxidant MnTBAP. *P.E. Coskun¹, S.E. Levy¹, B.J. Day², J.D. Crapo², D.C. Wallace¹*. 1) Center for Molecular Medicine, Emory University, Atlanta, GA; 2) National Jewish Medical and Research Center, Denver, CO.

The inactivation of the mitochondrial superoxide dismutase (Sod2) in mice results in a severe phenotype including dilated cardiomyopathy, fatty liver, OXPHOS defects and early neonatal death. These findings indicated that mitochondrial reactive oxygen species (ROS) are particularly deleterious to health. Previously, we demonstrated that the administration of the synthetic superoxide dismutase mimetic 5,10,15,20-tetrakis(4-benzoic acid)porphyrin (MnTBAP) prevents the cardiac and liver pathology and extends the survival rate. However, after 12 days the MnTBAP-treated animals develop a prominent movement disorder which leads to debilitation by three weeks, in association with spongiform changes and gliosis in the cortex and specific brain stem nuclei associated with motor function. The severe neuropathology probably results from the poor exchange of MnTBAP across the blood brain barrier. To investigate the effects of excessive mitochondrial ROS on gene expression in the brain, we have compared mRNA levels of wild type and MnTBAP-treated Sod2 animals using our mitochondrial gene DNA microarray (Mitochip) of 453 mouse cDNAs. Gene expression was profiled in 3 different groups, 8 day old without MnTBAP treatment, 8 day old with MnTBAP treatment and 12 day old with MnTBAP treatment. About 20 genes were found to be differentially expressed in all three groups of knockout mice compared to their age-matched controls. These included bioenergetic genes such as the mitochondrial creatine phosphokinase, antioxidant enzymes like glutathione peroxidase 3, and apoptotic factors including caspase 1 and apoptosis inhibitor factor 3. The excitatory amino acid transporter 3, frataxin and one EST of unknown function were also induced. Our data indicate that excessive amount of mitochondrial ROS toxicity affects mitochondrial gene expression of the brain, even at 8 days when the neuropathologic changes are not manifested. In addition, the expression of several of these genes was further perturbed in animals sustained using MnTBAP, indicating that the oxidative stress effects on the brain are cumulative.

Biochemical, Functional Characterization, and Clinical Implications of the Common Polymorphic Variants of SCAD. *T.V. Nguyen*¹, *C. Riggs*², *D. Babovic-Vuksanovic*¹, *N. Gregersen*³, *J. Vockley*¹. 1) Dept. Medical Genetics, Mayo Clinic, Rochester, MN; 2) Human Performance Laboratory, Univ. Arkansas, Fayetteville, AR; 3) Research Unit for Molecular Medicine, Aarhus Univ. Hosp. & Fac. Health Sciences, Skejby Sygehus, Denmark.

Short chain acyl-CoA dehydrogenase (SCAD) is a homotetrameric flavoenzyme that catalyzes the first intra-mitochondrial step in the β -oxidation of fatty acids. Two polymorphisms in the coding region of the SCAD gene: 511C>T (R147W) and 625G>A (G185S) have been shown to be associated with increased ethylmalonic acid excretion in urine, a clinical characteristic of a deficiency of SCAD. To characterize the biochemical consequences of these polymorphisms, in vitro site-directed mutagenesis and prokaryotic expression were used to produce the variant SCAD's. The variant enzymes were unstable when produced in *E. coli*, but could be stabilized and subsequently purified by co-expressing with the bacterial chaperonin protein GroEL/ES. SCAD's were purified to homogeneity using a combination of DEAE-Sephadex chromatography, ammonium sulfate fractionation, and chromatography on high-pressure ceramic hydroxyapatite. The k_{cat}/K_m values of wild type, reduced wild type, wild type co-expressed with GroEL/ES, R147W, and G185S determined with butyryl-CoA as the substrate were 1956, 1765, 2044, 1915 and 599 $\text{mM}^{-1} \text{min}^{-1}$, respectively. Spectrophotometric binding studies indicated that both of the variant proteins could effectively establish the charge-transfer enzymatic reaction intermediate. In contrast, near-UV circular dichroism (CD) studies revealed significant alterations in the observed spectrum of the variant enzymes at 292 and 309 nm. Alterations of the CD spectrum at these wavelengths indicated a reduced ability of variant enzymes to undergo conformational changes necessary to release electrons from the intermediate charge transfer complex to an electron acceptor, completing the enzymatic reaction. Finally the pH- and thermal stability of the G185S variant enzyme were significantly reduced compared to wild type. These results suggest that the common SCAD polymorphisms may lead to clinically relevant alterations in enzyme function..

Mitochondrial oxidative stress results in a decline of mitochondrial function and increased apoptosis with age in the Sod2^{+/-} mouse. *J.E. Kokoszka, P. Coskun, S.E. Levy, L.E. Esposito, D.C. Wallace.* Ctr for Molec Med, Emory Univ, Atlanta, GA.

Oxidative stress has been implicated in aging and degenerative disease. Mitochondria are thought to be the main source of reactive oxygen species such as superoxide anion. Mitochondrial superoxide anion is normally detoxified by manganese superoxide dismutase (MnSOD, Sod2). However, when free radical metabolism is perturbed, oxidative damage to protein, DNA, and lipids may occur. We have investigated the effects of increased superoxide anion toxicity on mitochondrial physiology with age in mice with a 50% reduction in MnSOD, Sod2^{+/-} mice. Animals were examined at young (5 months), middle (10-14 months), and old (20-25 months) ages. The control animals exhibited a linear decline in mitochondrial physiology with age. Mitochondria isolated from Sod2^{+/-} animals exhibited a decreased mitochondrial membrane potential, reduced power output, and increased predilection to mitochondrial permeability transition, a precursor to apoptosis. Middle aged Sod2^{+/-} animals also showed reduced respiratory control ratios and increased oxidized lipids, but these deficiencies returned to normal in the old animals. The predilection to the permeability transition, and the reversal of some respiratory defects in old Sod2^{+/-} animals, suggest that cells with severe mitochondrial damage might be removed by apoptosis. This was confirmed by TUNEL analysis of liver which revealed 4 times more TUNEL positive hepatocytes in the old Sod2^{+/-} than in age-matched controls. To better understand the molecular basis of these changes, we used our Mitochip to analyze changes in the expression of 453 genes involved in energy metabolism, oxidative stress, and apoptosis. Some notable changes in the old Sod2^{+/-} animals were the induction of antioxidant and apoptosis genes including glutathione peroxidase 3, apoptosis inhibitory factor 3, caspase 1, and the peripheral benzodiazepine receptor. Our data suggests that mitochondrial oxidative stress reaches a threshold in the late middle aged Sod2^{+/-} animals, initiating apoptosis which removes the cells with the most severely damaged mitochondria.

Clinical spectrum of muscle Coenzyme Q10 deficiency. *O. Musumeci, M. Hirano, A. Naini, S. Shanske, S. DiMauro.* Neurology, Columbia University, New York, NY.

Only a handful of patients have been described with muscle Coenzyme Q10 (ubiquinone) deficiency: this mitochondrial encephalomyopathy is characterized by the triad (i) recurrent myoglobinuria; (ii) central nervous system involvement (seizures, ataxia, or mental retardation); (iii) ragged-red fibers (RRF) and lipid storage in the muscle biopsy. However, the clinical spectrum associated with muscle COQ10 deficiency is probably wider. We now report nine patients with markedly decreased COQ10 levels in muscle (< 8.8 g/gm tissue, normal range 24-35) who fell into major clinical groups. Six patients had predominantly CNS involvement, with cerebellar ataxia, pyramidal signs, and seizures; there was weakness but no myoglobinuria, and muscle biopsy showed nonspecific changes. Brain MRI showed severe cerebellar atrophy. Two patients had exercise intolerance, recurrent myoglobinuria, and mild proximal weakness; muscle biopsies showed no RRF. One patient had both ataxia and myoglobinuria and her muscle biopsy showed lipid storage. Three of nine patients were siblings, both sexes were equally affected, and parents were asymptomatic, suggesting autosomal recessive transmission. All patients responded to COQ10 supplementation (300-1000 mg/day): strength increased, ataxia improved, seizures became less frequent. It is unclear whether these diverse clinical presentations reflect different biochemical or molecular defects in CoQ10 biosynthesis or different degrees of severity of a single biochemical defect. Irrespective of etiology, it is important to measure COQ10 concentration in muscle not only from patients with unexplained recurrent myoglobinuria but also from patients with autosomal recessive forms of ataxia and cerebellar atrophy, especially in view of the positive response to CoQ10 replacement.

Activated caspase-9 and -3 were involved in apoptosis of myogenic cell lines of MELAS. *M. Mori, S. Saitoh, T. Yamagata, M.Y. Momoi.* Department of Pediatrics, Jichi Medical School, Minamikawachi, Tochigi, Japan.

Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) is a multi-systemic disorder caused by a base substitution in the mitochondrial tRNA-Leu (UUR) gene. Mitochondrial genomes of the patients with MELAS are heteroplasmic with wild-type and mutated allele. We previously reported that dichloroacetate (DCA) that enhances the metabolism of pyruvate was effective for some symptoms of MELAS such as stroke, headache and cognitive functions. However, the effects for the long-course prognosis of this disease is unknown. To understand the cytological events that underlies the persistently progressive nature of the disease, the involvement of apoptotic process in this disease has to be studied. Although much has been revealed about apoptotic process and mitochondria, the involvement of apoptosis in mitochondria disorders is not clearly shown. We used our myogenic cell lines of MELAS to prove the presence of apoptotic process via activation of caspase-3. TUNEL positive apoptosis was induced by culturing cell in glucose-deficient medium. The time course of apoptosis was studied on cell lines with the A to G mutation of varying degree (0 to 80% of the mutated mtDNA), and the activation of caspase-9, -3 and the release of cytochrome c were studied by immunocytochemistry using specific antibody to each. Under the condition described above, only cell lines 80% mutated mtDNA were induced to TUNEL-positive cell death, that was almost compatible to our clinical observation. Some cells were positive for activated caspase-9 and caspase-3 in cell lines with 80% mutated genome, and cytochrome c was detected diffusely in cytoplasm in these cells. These showed that myogenic cells of MELAS is induced to apoptosis that was through the release of cytochrome c and the activation of caspase-9 and -3.

Endothelial Dysfunction in patients with A3243G mutation in the mitochondrial tRNA^{Leu(UUR)} gene. *Y. Koga¹, H. Matsuoka², M. Ishibashi³, Y. Akita¹, T. Imaizumi², H. Kato¹.* 1) Dept Pediatrics & Child Health,; 2) Department of Internal Medicine III,; 3) Department of Radiology, Kurume Univ Sch Medicine, Fukuoka, Japan.

MELAS characterized by stroke before 20 years old have been reported to have an A3243G mutation in the mitochondrial tRNA^{Leu(UUR)} gene. Mitochondrial angiopathy demonstrating degenerative change with increased abnormal mitochondria in the endothelial cells of intramuscular small arteries and arterioles has been reported in many MELAS patients. In this study, we examined flow-mediated vasodilation, as a non-invasive measure of endothelial function, in patients with MELAS. We also studied the pharmacological effect on the clinical course, and biochemical parameters after administration of L-arginine to a patient in the acute phase of stroke on three separated occasions and, and on the functional aspects of the cerebral hemodynamics using single photon emission computed tomography (SPECT). All patients were fulfilled the clinical criteria of MELAS and were proven to have an A3243G mutation in the mitochondrial tRNA^{Leu(UUR)} gene. Flow-mediated vasodilation was significantly less in MELAS patients. Endothelium-dependent vasodilation induced by glyceryl trinitrate was also impaired. After the administration of L-arginine, all the symptoms of the patient suggesting the strokelike episode were clinically improved. On SPECT using ECD, the intracranial hemodynamics were also improved in the ischemic area (in the left temporal lobe), but unchanged in the brain stem (thalamus). Our data indicated that the L-arginine therapy improved the microcirculation to reduce tissue injury from ischemia, and therefore constitutes a new potential therapy for use in the acute phase of stroke like episodes in MELAS, especially in younger patient when atherosclerosis has not yet developed. Our data also demonstrated that angiopathy seen in MELAS involved abnormality in the capacity of vasodilatation in the endothelial system, which may play an important role in causing strokelike episodes in this disorder.

Two novel mutations in mtDNA structural genes. *V. Tiranti¹, P. Corona¹, M. Greco¹, J.W. Taanman², F. Carrara¹, M. Zeviani¹.* 1) Div Biochem & Genetics, Istituto Nazionale Neurologico, Milano, Italy; 2) Royal Free Hospital, London, UK.

We report here two new mutations of mtDNA affecting structural genes. The first is a heteroplasmic mutation in the mtDNA gene encoding subunit ND5 of complex I. This mutation hits the same D393 amino acid residue affected by a previously reported MELAS-associated mutation. However, it leads to a different amino acid replacement (D393G vs. D393N). The mutation was found in two unrelated patients, both affected by a MELAS-like syndrome. Our results clearly demonstrate that the amino acid position D393 is crucial for function of complex I. Changes in this position can be a relatively frequent cause of MELAS or other mitochondrial phenotypes, suggesting that search for D393 mutations should be part of the routine screening for these disorders. The second mutation is a virtually homoplasmic C insertion causing a frameshift in the mtDNA ORF for COX subunit III. The proband is a 10-year old girl with a negative family. Since 4 years of age she developed progressive spastic paraparesis, associated with ophthalmoparesis, convergent strabismus, reduced visual acuity, and moderate mental retardation. The presence of severe lactic acidosis and Leigh-like lesions of putamina prompted us to perform muscle and skin biopsies. In both, a profound, isolated defect of COX was found by histochemical and biochemical assays. In vivo mtDNA translation and Western-blot analyses clearly demonstrated the complete absence of COX III protein. The role of this subunit is controversial. Experiments on simpler organisms seem to exclude an important function in the catalytic activity of complex IV. However, COX III could play a crucial role as an assembler of COX. To test this hypothesis, we performed western-blot analysis using antibodies against individual COX subunits on patients mitochondrial proteins separated by 2D blue-native electrophoresis. Results showed an accumulation of early-assembly intermediates of COX, while the fully assembled complex was absent. Several of the smaller subunits of COX were missing, suggesting that COX III is indeed essential for the assembly of a structurally complete and functionally active enzyme.

CLONING OF A cDNA FOR HUMAN PYRUVATE DEHYDROGENASE PHOSPHATASE AND DETECTION OF A MUTATION IN A PATIENT WITH CONGENITAL LACTIC ACIDEMIA. *K. Shinahara¹, I. Ohigashi¹, M. Ito¹, Y. Ogawa¹, N. Bando¹, T. Saijo¹, I. Yokota¹, E. Naito¹, M. Sone², S. Furukawa², Y. Kuroda¹.* 1) Dept Pediatrics, Univ Tokushima, Tokushima, Japan; 2) National Kagawa Children's Hospital, Zentsuji, Japan.

Pyruvate dehydrogenase phosphatase (PDP) is one of the few mammalian phosphatase residing within the mitochondrial matrix space. A defect of activation of pyruvate dehydrogenase complex (PDC) causes congenital lactic acidemia and PDP is responsible for activation of PDC. However, the molecular genetical analysis of PDP in a patient with congenital lactic acidemia has not been performed as yet. In this study we cloned two cDNA encoding the catalytic subunit of human PDP (PDP1, PDP2), and detected a mutation in a patient with a defect of activation of PDC. Human PDP1 and PDP2 are highly homologous to the previously identified rat PDP1 and PDP2 in level of nucleotide and protein. Furthermore two genes (PDP1 and PDP2) were mapped in chromosome 8, in the region q22-23, and in chromosome 3, in the region q27-28, respectively. When DNA for PDP2, which from patient with congenital lactic acidemia due to defect of activation of PDHC, were sequenced and a A to T substitution at nucleotide 716, which alters D 239 to V was observed. This amino acid was conserved in human and previously reported PDP1 and PDP2. These results suggest that this mutation is the primary defect of activation of PDHC in this patient.

Molecular basis of mucopolysaccharidosis type IIIB in emu (*Dromaius novaehollandiae*), first animal model of Sanfilippo syndrome type B. *E.L. Aronovich¹, J.M. Johnston¹, P. Wang², U. Giger², C.B. Whitley¹.* 1) Department of Pediatrics, and Institute of Human Genetics, University of Minnesota, Minneapolis, MN, USA; 2) Section of Medical Genetics, University of Pennsylvania, Philadelphia, PA, USA.

Sanfilippo syndrome type B, or mucopolysaccharidosis (MPS) IIIB, is an autosomal recessive disease caused by deficiency of lysosomal alpha-N-acetylglucosaminidase (NAGLU). A progressive neurological disease in *Dromaius novaehollandiae* (i.e., emu) has been associated with ganglioside accumulation; the disorder was recently discovered to be due to NAGLU deficiency with heparan sulfate accumulation (Giger et al, Vet Pathol 14:5, 1997). Initial attempts to characterize the molecular pathology based on available human and murine NAGLU sequences failed; however, additional information from the tobacco gene facilitated design of "interspecies primers" that allowed amplification of avian cDNA fragments. Resultant RT-PCR and 5'- and 3'-RACE sequence fragments were computer-assembled into a cDNA with an open reading frame (ORF) of 2,259 bp. The sequences of the introns were subsequently determined. The emu NAGLU gene is structurally similar to that of man and mouse, but the introns are considerably shorter. The deduced amino acid sequence shares 64% identity with human, 63% with mouse, 41% with drosophila, 39% with tobacco and 35% identity with the *C. elegans* NAGLU enzyme. Three normal and two affected birds were studied for nucleotide sequence covering the entire coding region and exon-intron boundaries. In contrast to the human gene, the emu NAGLU gene was found to be highly polymorphic; 19 variations were found in the coding region alone. Notably, two affected birds were found to be homozygous for a 2 bp deletion. The resulting frameshift would yield a longer ORF of 2,370 bp encoding a polypeptide with 37 additional, and 387 altered, amino acids. Availability of mutation analysis in emu now permits detection of NAGLU mutations in breeding stocks, and is an important step in characterizing this unique naturally-occurring animal model for the development of gene transfer studies. Support: Childrens' Medical Research Foundation.

Characterization of the Canine SLC3A1 Gene and Identification of a Nonsense Mutation in Cystinuric Newfoundland Dogs. *P.S. Henthorn, J. Liu, T. Gidalevich, M.L. Casal, P. Werner, M.G. Raducha, U. Procuik, D.F. Patterson, U. Giger.* Center for Comparative Medical Genetics, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

Cystinuria is an inherited renal disease characterized by defective amino acid reabsorption and by the formation of cystine uroliths. Different forms of the disease, designated type I and non-type I, can be distinguished clinically and biochemically, and have been associated in cystinuric humans with mutations in the SLC3A1 (rBAT) and SLC7A9 genes, respectively. Cystinuria is primarily inherited as an autosomal recessive trait in humans.

Cystinuria has been recognized in over 70 breeds of dogs and a severe form, resembling type I cystinuria, has been characterized in the Newfoundland breed. We have isolated and sequenced the canine SLC3A1 cDNA and gene from normal and cystinuric Newfoundland dogs. Affected Newfoundland dogs are homozygous for a nonsense mutation in exon 2 of the gene that truncates the predicted SLC3A1 protein to less than one third of its normal length. A DNA-based test was developed to detect carrier animals for use in eliminating this disease from the Newfoundland dog population. FISH studies indicate that the canine SLC3A1 gene is located on canine chromosome 10.

The involvement of the SLC3A1 gene was examined in other dog breeds. In six other breeds, either heterozygosity at the SLC3A1 locus or lack of mutations in the coding region of the SLC3A1 gene, indicates that cystinuria is genetically heterogeneous in dogs with possible involvement of other gene loci, as in humans.

In addition to providing a test for the diagnosis and control of cystinuria in Newfoundland dogs, these studies demonstrate a true homology with human type I cystinuria. A breeding colony of dogs with the disease provides the opportunity to use a large animal model to investigate molecular approaches for the treatment of cystinuria and other renal tubular diseases.

Albright Hereditary Osteodystrophy (AHO) and Pseudohypoparathyroidism: three new mutations and a common deletion in GNAS1. *L. de Sanctis¹, D. Romagnolo¹, C. de Sanctis¹, R. Lala¹, M. Olivero², F. Di Renzo², I. Dianzani³.* 1) Osp.Inf. Regina Margherita, Torino, Torino, Italy; 2) IRCC, Univ.Torino, Torino, Italy; 3) Dip. Scienze Mediche, Univ. Piemonte Orientale, Italy.

Pseudohypoparathyroidism (PHP) (OMIM 300800) is a heterogeneous disease characterized by parathormone (PTH) resistance and classified in type Ia, Ib, Ic and II, depending on the different pathogenetic mechanism and phenotype. Patients with PHP type Ia (PHP-Ia) show G_sa protein deficiency, PTH resistance and Albright Hereditary Osteodystrophy (AHO). Heterozygous mutations in GNAS1 gene, encoding G_sa protein, have been identified in PHP-Ia, but also in PseudoPHP, a disorder with isolated AHO. Within the same family a single GNAS1 mutation may be responsible for PHP-Ia when inherited from the maternal allele and for PseudoPHP when inherited from the paternal allele, suggesting that GNAS1 is an imprinted gene. We performed GNAS1 mutational analysis in 14 AHO patients with PTH resistance from 12 unrelated Italian families. Direct sequencing of exons 2-13 from genomic DNA allowed to identify 4 mutations in 5 patients and a common polymorphism (I131I). Three mutations (P115L, delC185 and R336W) are new mutations, the fourth (del4bp in exon 7) is the most common mutation reported in GNAS1. All mutations are derived from the mothers, that displayed an isolated AHO phenotype (PseudoPHP). These data allow to make a definite diagnosis of PHP-Ia and PseudoPHP in the mutated patients and evidence that different causes are responsible for PHP. Moreover, GNAS1 molecular analysis shows mutation heterogeneity for G_sa protein deficiency in the Italian population, confirms the variable expressivity for GNAS1 mutations, which are associated either with PHP-Ia or PseudoPHP, and further suggests genomic imprinting to explain the occurrence of both diseases within the same family.

Identification of a new RNA splicing defect and a new point mutation in OTC gene. *S. Bisanzi¹, A. Morrone¹, S. Di Donato², P. Strisciuglio³, E. Zammarchi¹.* 1) Department of Pediatrics, University of Florence; 2) Neurological Institute C.Besta, Milan; 3) Dept of Pediatrics-Univ of Reggio Calabria, Italy.

Ornithine transcarbamylase deficiency (OTCD) is an X-linked inborn metabolism error of the urea cycle; it is the most common urea cycle disorder and is due to a defect of the mitochondrial enzyme ornithine transcarbamylase (OTC), which is expressed specifically in liver and gut. The OTC gene is subject to heterogeneous mutations, which may lead to partial or complete enzymatic deficiency. Clinical presentation is highly variable, usually more severe in newborn male patients, who frequently present early symptoms with marked hyperammonemia, lethargy, seizures, hypotonia, coma and often exitus at neonatal age. In hemizygous male patients who present varying degrees of severity, which is principally correlated to different mutations and enzyme activity, and in heterozygous females who are manifesting carriers, the onset of the disease can also occur later. Manifesting carriers present a clinical phenotype ranging from asymptomatic to severe hyperammonemia, depending on X-inactivation. In two OTCD patients, a manifesting carrier and a male patient, two new genetic lesions in the OTC gene were identified. Molecular analysis by direct sequencing of OTC cDNA, genomic DNA and enzymatic restriction analysis were performed on the patients' genomic DNA and total RNA isolated from peripheral blood lymphocytes. A new transversion IVS1-3C>G was identified in the manifesting carrier's genomic DNA. The patient's cDNA analysis revealed two transcripts: the normally spliced transcript and an aberrant transcript with an AG insertion. This insertion was due to the identified nucleotide change C>G which generates a new acceptor splice site (AG) in intron 1; this induces an RNA splicing defect with the insertion of two nucleotides between exon 1 and exon 2 (c77-78ins2nt) in OTC mRNA. This insertion causes a frame shift in OTC cDNA ORF and then leads to a stop codon. In the male patient a new amino acid substitution S132P due to nucleotide change c394T>C was identified. Molecular analysis of this patient's family identified as manifesting carriers the proband's mother, sister, aunts and a cousin.

Prenatal diagnosis of *ALPL* G317D/V365I mutations in a fetus with lethal hypophosphatasia: Pitfalls and Implications. S.M. Nikkel, C.R. Greenberg, A.E. Chudley, D. Ramadeen, E. Grudeski, R. Singal, E.L. Spriggs. Health Sciences Centre and University of Manitoba, Winnipeg, MB, Canada.

Hypophosphatasia is an autosomal recessive disorder of bone mineralization and deficient activity of the tissue non-specific alkaline phosphatase (TNSALP) isoenzyme. We have previously reported homozygosity for *ALPL* 1177G®A (G317D) mutation as the cause of lethal hypophosphatasia in Canadian Mennonites (*Am J Hum Genet* 1992 **51**:A169). We now describe the DNA results in a fetus who was suspected to have hypophosphatasia based on ultrasound findings and biochemical parameters suggestive of carrier status in the parents. The mother is of Mennonite background and the father is of Anglo-Scottish descent. Using our allele-specific-oligonucleotide (ASO) hybridization method for detection of the G317D mutation (*Genomics* 1993 **17**:215), the mother was found to be a carrier for the G317D mutation. The fetus appeared to be homozygous for G317D, while the father appeared to be homozygous for the wild type allele. The possibility of non-paternity was ruled out. New primers were used in a PCR reaction that flanked the PCR product amplified in the original ASO detection method. This was followed by a restriction digestion of the PCR product with BsmAI. An extra BsmAI site is created in the presence of *ALPL* 1177G®A. Mother and fetus were now found to be heterozygous for the G317D mutation, and the father had two wild type alleles. Sequencing of the PCR products from the BsmAI method showed that the father and fetus were heterozygous for an *ALPL* 1320G®A (V365I) mutation. This V365I mutation was within the reverse primer sequence used in the original ASO detection method. Thus an apparent “null” allele was created in the father and fetus when the ASO method was used. The V365I mutation has been described once in a Japanese patient who had childhood onset hypophosphatasia, the second mutation being F310L. Our patient shows that compound heterozygosity for the V365I mutation with another known severe mutation can cause lethal hypophosphatasia. Our experience also demonstrates that mutations within the primer sequences used in PCR reactions must be considered when unexpected results are obtained.

Mutational analysis of ATP7B gene in Chinese patients with Wilson disease. *X. Liu, Y.F. Zhang, R.G. Chen, X.F. Gu.* Shanghai Second Medical Univer, Shanghai, China.

OBJECTIVE ATP7B gene was analysed in 58 Chinese patients with Wilson disease (WD), a genotype and phenotype correlation were studied. **METHODS** Exon 1~21 of ATP7B gene mutations was conducted in 58 WD patients from 54 no kinship family by using PCR-SSCP and DNA sequence analysis. **RESULTS** 50/58 patients were presented an hepatic symptom (86.21%), 12/50 patients were presented both hepatic damage and neurological dysfunction (24%), 10/50 patients were presented hepatic damage and other symptom (20%); 7/58 patients were presented only neurological dysfunction (12.07%), one was an asymptomatic patient. 19 of 21 exons were displayed an abnormal pattern on SSCP analysis. Ten mutations were identified, those are four missense mutations R778L, G943S, V1106I, V1216M, one deletion 1384del17 and five polymorphisms IVS4-5T/C, K/R832, L/L770, IVS18+6C/T, IVS20+5A/G. 52/116 allele were R778L with a frequency of 44.83%. The frequency of V1106I was 1.72% in this study, two patients carry this mutation are late onset phenotype. **CONCLUSION** 1384del17bp and V1106I are two novel mutations. G943S and V1216M were firstly identified in Chinese WD patients. The mutation R778L in exon 8 of WD gene may play an important role in pathogenesis of Wilson's disease in Chinese.

Molecular genetic analysis of patients with glycogen storage disease type Ia (GSD Ia). *D. Matern*^{1,2,3}, *D. Bali*², *H.H. Seydewitz*³, *Y.T. Chen*². 1) Division of Laboratory Genetics, Mayo Clinic, Rochester, MN; 2) Division of Medical Genetics, Duke University, Durham, NC; 3) Children's Hospital, Albert-Ludwigs-University, Freiburg, Germany.

BACKGROUND: The gene for the phosphatase (G6PC) of the microsomal glucose-6-phosphatase system (G6Pase) spans 12.5 kb on chromosome 17 and consists of 5 exons. G6PC mutations cause GSD Ia, which is characterized by hepatomegaly, hypoglycemia, lactic acidemia, hyperuricemia, hyperlipidemia and short stature. So far the G6Pase enzyme assay in a liver biopsy specimen is considered the gold standard for verification of the clinical diagnosis of this autosomal recessive disorder. We report the molecular genetic analysis of G6PC in 111 GSD Ia patients of various ethnic backgrounds. **METHODS:** DNA was isolated from EDTA-blood of 111 GSD Ia patients. All 5 exons and the promoter region of G6PC were screened for mutations by SSCP analysis following PCR amplification. Mutations found by SSCP analysis were further characterized by DNA sequencing. In the event of normal SSCP band-patterns, all exons and the promoter region were sequenced for 96 patients. **RESULTS:** 30 mutations were identified on 207 alleles. No mutation was detected on 15 alleles (for 2 patients no mutation was detected by SSCP and 11 patients were heterozygous for 1 mutation), however, DNA sequencing has not yet been performed on these samples. For another 15 patients, the clinical diagnosis of GSD Ia was confirmed by mutation analysis. No true common mutation was identified, with the mutations R83C and Q347X occurring in this heterogenous study population with the highest frequencies of 37% and 19%. Homozygosity for the G188R mutation in 5 patients was associated with mild neutropenia and recurrent infections, a phenotype more consistent with GSD Ib than GSD Ia. **CONCLUSION:** GSD Ia is a genetically heterogenous disease. This study suggests that molecular genetic analysis can be a reliable and convenient alternative to the enzyme assay in a fresh liver biopsy specimen to diagnose GSD Ia, when DNA sequencing is utilized to follow up on uninformative SSCP analysis. A mild GSD Ib phenotype is associated with homozygosity for the G188R mutation.

Identification of a novel 200-kb deletion spanning the 5'-end of the aspartoacylase gene in a family with Canavan disease. *F.E. Tahmaz, G.E. Hoganson, F. Quan.* Dept Pediatrics, Div Genetics, Univ of Illinois at Chicago, Chicago, IL.

Canavan disease is an autosomal recessive leukodystrophy caused by the accumulation of N-acetylaspartic acid (NAA) in the brain as a result of a deficiency of aspartoacylase (ASPA) activity. The spectrum of mutations in the ASPA gene causing Canavan disease includes point mutations and small insertions and deletions (1-4 bp). Large deletions of the ASPA gene have not previously been described. We report here a Mexican family in which 2 children were affected with Canavan disease as a result of homozygosity for a deletion of approximately 200 kb. This deletion removed the first 2 exons of the ASPA gene. The parents were second cousins once removed. Using DNA samples from the affected children, introns 1 and 2 of the ASPA gene could not be amplified in PCR reactions with primers located in the flanking exons. The presence of a deletion was confirmed by Southern blot analysis using a probe from the 3'-end of intron 2. With different restriction enzymes, this probe identified junction fragments spanning the region of the deletion breakpoint in the ASPA gene. A 2.1 kb BamHI junction fragment was isolated using an inverse-PCR strategy. Sequence analysis demonstrated that the proximal breakpoint of the deletion was in intron 2, approximately 500 bp upstream of exon 3. The distal deletion breakpoint was found to be in a MER-2 repetitive element located approximately 20-kb downstream of the Olfactory Receptor (OR) gene cluster on chromosome 17p13.3 (Glusman et al [2000] *Genomics* 63, 227-245). Based on a previously reported YAC contig containing both the ASPA gene and the OR gene cluster (McDowell et al [1996] *Biochem Mol Med* 58, 135-141), we estimated that the size of the deletion was approximately 200-kb. This is the first report of a large deletion spanning part of the ASPA gene in Canavan disease. This demonstrates that the spectrum of mutations that lead to Canavan disease must be expanded to include deletions of the gene. Therefore, the possibility of an ASPA gene deletion should be considered in those patients in whom mutations in both alleles can not be identified.

Mutations in CYP11B1 and congenital adrenal hyperplasia in Moroccan Jews. *T.Y. Paperna¹, L. Kasinetz¹, Z. Hochberg², R. Gershoni-Baruch^{1,2}.* 1) Institute of Genetics; 2) Department of Pediatrics, Rambam Medical Ctr., Haifa, Israel.

Steroid 11 β -hydroxylase (11-OH) deficiency is the second major cause of congenital adrenal hyperplasia (CAH) with a reported incidence of 1:100,000. In Jews of Moroccan (MJ) descent, the prevalence of 11-OH deficiency is much higher and was estimated at 1:5000-1:7000. This implies a high carrier frequency of ~1:40. In a previous report, a single mutation in the CYP11B1 gene (encoding 11-OH), R448H, was suggested to account for the majority of disease alleles in this population (>90%). We determined the prevalence of the R448H mutation in 209 Israeli MJ, none of which reportedly had any known personal or family history of CAH, and detected only one carrier. In 13 unrelated MJ patients referred for CAH due to 21-hydroxylase (21-OH) deficiency, two R448H heterozygotes were found. Of these, one was homozygous for a known 21-OH mutation while in the other none of 8 common 21-OH mutations was found. Out of two MJ patients, diagnosed with 11-OH deficiency, one was homozygous for the R448H mutation while the other was a compound heterozygote for R448H and R448C. This report is the first to determine R448H carrier rate in the general MJ population, a rate lower than previously suggested. Previously estimated disease incidence may be attributed to the concurrence of two different mutations in the same codon in MJ patients.

Mutation analysis of type 2 Gaucher disease in Taiwan Chinese and identification of two novel mutations. *F.J. Tsai¹, J.Y. Wu¹, S.P. Lin², J.G. Chang¹, C.C. Lee¹, C.H. Tsai¹.* 1) Dept Pediatrics, China Medical Col Hosp, Taichung, Taiwan; 2) Dept Pediatrics, Mackay Memorial Hospital, Taipei.

Gaucher disease (GD), one of the most prevalent lysosomal storage diseases, is caused by deficiency of lysosomal acid beta-glucosidase (GBA). It is divided into three types according to the presence and progression of neurologic symptoms. Of those three types, type 2 is relatively rare and most severe; patients usually died before age of two years. Molecular analysis of GBA gene in five type 2 GD patients of Taiwan Chinese decent identified two novel mutations: G355E and three-nucleotide insertion in exon 7 of GBA. The latter resulted in a methionine residue insertion between residues 241(Leu) and 242 (Ser). L444P, the second most common GD mutation among non-Jewish Caucasian population, was found in all five type 2 GD patients (50%) in this study. Overall 9 out of 10 GD alleles has been identified. This report is the first mutation study focusing on type 2 GD in Taiwan Chinese. The identified new mutations further delineate genetic heterogeneity in the lesions causing type 2 GD, and further provides molecular insights in genotype/phenotype correlation and in functional domains sketching within the acid beta-glucosidase.

Identification of cathepsin C mutations in ethnically diverse PLS patients. *Y. Zhang¹, P.S. Hart¹, E. Firatli², C. Uygun², M. Lotfazar³, M.D. Michalec¹, J.J. Marks¹, X. Lu¹, B.J. Coates¹, W.K. Seow⁴, R. Marshall⁴, D. Williams⁵, J.T. Wright¹, T.C. Hart¹.* 1) University of Pittsburgh, Pittsburgh, PA; 2) University of Istanbul, Istanbul, Turkey; 3) Shiraz University of Medical Sciences, Iran; 4) University of Queensland, Brisbane, Australia; 5) The Leicester Royal Infirmary, England.

Papillon Lefevre syndrome (PLS) is an autosomal recessive disorder characterized by palmoplantar keratoderma and aggressive periodontitis. The condition results from deficiency of cathepsin C (CTSC) activity secondary to mutations in the CTSC gene. The generality of CTSC mutations was evaluated in an ethnically diverse group of PLS patients from 20 unrelated families. Mutations were identified by direct automated sequencing of genomic DNA amplified for exonic regions and associated splice site junctions of the CTSC gene. A total of 15 different mutations in exons 2-7 were identified in affected individuals from 14 families. Eleven mutations were novel and 4 have been reported previously. Missense mutations were most common (9/15), followed by nonsense mutations (3/15), insertions (2/15), and deletions (1/15). Two probands were compound heterozygotes for different CTSC mutations, all other probands were homozygous for a given mutation, reflecting consanguinity. CTSC, a member of the papain family, is a lysosomal cysteine proteinase with main function of protein degradation. CTSC enzymatic activity was evaluated in a variety of primary and established cell lines by fluorescent substrate cleavage. Enzyme activity was highest in primary placental fibroblasts and leukocytes, while least activity was detected in breast fibroblasts. CTSC activities of leukocytes from normal, carrier, and affected individuals were consistent with their genotype with relative activity of wt/wt, ~100%, wt/mut, ~50%, and mut/mut, less than 10%. There was no evidence of differing phenotype corresponding to any particular CTSC mutation. A comprehensive list of all CTSC mutations described to date, representing a total of 25 mutations from 32 families, is presented. The gene spans over 46 kb, with six introns ranging in size from 1.6 to 24.4 kb.

Analysis of the heparin sulfamidase gene: mutation analysis in patients with Sanfilippo syndrome type A (MPS IIIA) and analysis of sulfamidase expression in normal adult brain. *T.C. Wood, J.N. Thompson.* Dept Human Genetics, Univ Alabama at Birmingham, Birmingham, AL.

Sanfilippo syndrome, type A (MPS IIIA, MIM 252900) is a genetic lysosomal storage disease in which patients are distinguished by mild somatic features and degenerative neurological problems, including a wide spectrum of behavioral disturbances. MPS IIIA is characterized by a deficiency in heparin sulfamidase that results in defective degradation of heparan sulfate and heparin. The faulty degradation manifests itself in the continuous accumulation of partially degraded polysaccharide in the tissues of patients and increased polysaccharide excretion in the patient's urine. Recent isolation and characterization of the sulfamidase gene has allowed for the identification of mutations in the gene. To date, over 50 unique mutations have been described in European and Australian populations, however the number of U.S. individuals studied remains small. In the present investigation, twenty-four different mutations were found in 39 U.S. MPS IIIA patients. Five of these mutations were previously unreported - Y286S, R433W, 1164delC, 1389insC, 1184delCTTCAA. Five other mutations were found to have allele frequencies greater than or equal to 5 % - S66W (13 %), R245H (10 %), R74C (8 %), P293S (6 %), E447K (5 %). Carrier status was determined in three families in which each parent was found to be a carrier. In one family the mutations were traced back to the grandparents of the proband. RNA from total brain and cerebellar extracts showed sulfamidase expression using both northern blot and RT - PCR approaches. These results support data from the UNIGENE database (NCBI) that heparin sulfamidase is expressed in the brain.

Niemann-Pick Type A: unexpected frequency of the R496L, fsP330, and L302P mutations in the Ashkenazi Jewish population. *L.S. Rosenblum-Vos, E.A. Sugarman, B.A. Allitto.* Genzyme Genetics, Framingham, MA.

Niemann-Pick disease (NPD) is an autosomal recessive lysosomal storage disorder caused by mutations in the *ASM* gene that result in deficiency of acid sphingomyelinase. Two allelic forms of NPD, type A (NPD-A) and type B (NPD-B) disease, have been described based on their phenotypes. NPD-A has the more severe presentation, characterized in infancy by failure to thrive, hepatosplenomegaly and progressive neurodegeneration leading to death by three years of age. It is most prevalent among Ashkenazi Jewish individuals, with an estimated incidence of 1 in 30,000. Early studies reported a heterozygote frequency of 1/90. Three mutations in the *ASM* gene, R496L, fsP330, and L302P, accounted for 95% of the mutant alleles in the Ashkenazi Jewish population. In a small group of NPD-A patients and obligate carriers the R496L, fsP330, and L302P relative mutation frequencies were reported at 49%, 29%, and 25% respectively. In a screening study of 1000 Ashkenazi Jewish individuals these relative rates were reported at 82%, 18%, and 0% respectively. We have analyzed data collected as part of routine clinical molecular carrier testing to determine these frequencies in 3826 Ashkenazi Jewish individuals with no family history of NPD-A. Mutation detection was performed using PCR amplification and allele-specific oligonucleotide hybridization analysis. In contrast to previous reports, we identified 33 heterozygous positive individuals for a carrier frequency of 1/116 (0.9%) and a mutation distribution of 16 R496L (48%), 14 fsP330 (42%), and 3 L302P (9%). In summary, our data show that 1) the fsP330 mutation is as important as R496L, 2) all three NPD-A mutations should be included in a screening assay, and 3) it is necessary to continue monitoring the mutation frequencies for Niemann-Pick Type A disease.

Identification of a novel mutation in cystathionine beta-synthase (CBS) among American Blacks. *M.T. Steen*², *W.D. Kruger*¹, *L. Wong*¹, *R. Dunbrack*¹, *L.J. Elsas II*². 1) Fox-Chase Cancer Center, Philadelphia, PA; 2) Division of Medical Genetics, Emory University, Atlanta, GA.

Although homocystinuria (HCU) caused by CBS deficiency is relatively common in persons of European ancestry, little is known about HCU in persons of other ethnicities such as American Blacks. We developed methods for identifying mutations in twelve Emory patients with HCU, from eleven families. All sixteen exons of CBS were amplified by PCR and sequenced. Each suspected missense mutation was engineered into the human CBS cDNA and expressed in a CBS-null yeast auxotrophic for cysteine. Mutations were defined by the absence of growth on cysteine-free medium. All nine missense mutations exhibited auxotrophic phenotypes in recombinant yeast. A novel splice-acceptor mutation (G₇₃₆-1C) was suspected in a compound heterozygote, in trans with the G307S mutation, since only G307S-containing mRNA was detectable in lymphoblasts obtained from this patient. Three mutations were previously-described in European patients (I278T, G307S, L101P). Six novel missense mutations (A226T, N228S, A231L, T353M, D376N, Q528K) were proven in the yeast system. Four out of our twelve HCU patients are black and the novel T353M mutation was present exclusively in these black patients. The genotypes of our Black patients were T353M/T353M, T353M/A226T, T353M/Q528K, and T353M/unknown; only the patient with the T353M/A226T genotype clinically responded to pharmacologic B6 supplementation. We conclude that a methionine substitution for threonine at position 353 is a mutation in CBS that does not confer B6-responsivity, and that it is common in American Blacks.

Molecular analysis of the N-acetylgalactosamine-6-sulfate sulfatase gene in Taiwanese patients with

Mucopolysaccharidosis IVA. *C.F. Yang¹, F.J. Tsai^{1,2}, S.P. Lin³, J.Y. Wu^{1,2}*. 1) Department of Medical Research, China Medical College Hospital, Taichung, Taiwan; 2) Department of Medical Genetics, China Medical College Hospital, Taichung, Taiwan; 3) Department of Pediatrics, Mackay Memorial Hospital, Taipei, Taiwan.

Mucopolysaccharidosis IVA (MPSIVA) is an autosomal recessive lysosomal storage disorder resulting from a deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS). We studied mutations in the GALNS genes by using direct sequencing from 3 unrelated patients with a severe form of MPSIVA in Taiwan. A total of 1 novel deletion, 1 previously reported missense mutation, and 1 new polymorphism was identified. The novel mutation, 106-111delCTGCTC in exon1, results in an inframe deletion of 2 Leucine. The M318R mutation was identified previously and accounted for 4 of 6 unrelated alleles. Of the 3 patients with MPSIVA identified, one patients with unrelated parents was homozygous with the M318R mutation and 2 were heterozygous for 106-111delCTGCTC and M318R. A new polymorphism, T to C transition at nucleotide 708, was identified homozygous in all 3 patients. This is the first report of the molecular analysis of MPSIVA in Asian population.

HOLOCARBOXYLASE SYNTHETASE DEFICIENCY: CLINICAL FINDINGS AND IDENTIFICATION OF NEW MUTATIONS. *S. Malvagia¹, A. Morrone¹, E. Pasquini¹, M.A. Donati¹, I. Pela¹, S. Funghini¹, T. Bardelli¹, A. Boneh², H. Peters², E. Zammarchi¹.* 1) Dept. of Ped. Univ., Meyer Children's Hospital, Florence, Italy; 2) Victorian Clin. Gen. Serv, Murdoch Inst, Victoria-Australia.

Holocarboxylase synthetase (HLCS) is the enzyme that catalyses the biotinylation of the four human biotin dependent carboxylases. Its deficiency leads to biotin-responsive multiple carboxylase deficiency (MCD). We report the clinical and molecular studies of three HLCS patients (one Italian, one Iranian, one Australian). The Italian patient, at nineteen months, exhibited skin lesions over the entire body. The typical pattern of urinary organic acids led us to the diagnosis of MCD. The HLCS activity showed a Km for biotin of 94.8 nmol/l (n.v. 153). The clinical symptoms disappeared with biotin 10mg /die. At molecular analysis the patient was a compound heterozygous for two new amino acid substitutions: G582R and N511K. Both these amino acid substitutions are localized in the HLCS biotin-binding domain and the G582R is one of the eight conservative residues that form hydrogen bonds with the biotin. The Iranian patient died at the age of six months and diagnosis was made postmortem by organic acid and molecular analysis. He was homozygous for R508W amino acid substitution. The Australian patient, of Samoan/Mauri ethnic origin, presented in the neonatal period a severe metabolic acidosis. Biotin therapy didn't show any clinical improvement with biotin 20 mg/die, even after it was increased to 100 mg/die. Elevated levels of some organic acids were still present in his urine. Now, he is two years old and shows psychomotor development delay and severe skin eczema with infections. He was homozygous for L216R amino acid change located in the 5' of HLCS region. Earlier expression studies reported that this mutation led to a reduction of Vmax but not of Km. At the western blot analysis the expression level of this mutant HLCS protein was the same as that of the wild type protein. These data combined with the severe clinical phenotype and partial biotin responsiveness of our patient suggested that this region might be necessary for defining substrate specificity or regulation of this enzyme.

Alkaptonuria: Inhibition of Collagen Lysyl Hydroxylase is an Unlikely Cause of Ochronotic Arthritis and Arteriosclerosis. *B. Steinmann*¹, *P.R. Huber*². 1) Div Metabol & Molec Pediatr, CH-8032 Zürich, Switzerland; 2) University Hospital, CH-4031 Basel, Switzerland.

Alkaptonuria is a rare, autosomal recessive disorder caused by deficiency of homogentisic acid oxidase which leads to impaired cleavage of homogentisic acid (HGA) and to its accumulation in body fluids. Urine, cerumen, and sclerae may show a diagnostic darkening while HGA products lead to black ochronotic deposits in cartilage, heart valves and large arteries, and eventually result in severe arthritis and cardiovascular changes. It has been observed that collagen lysyl hydroxylase (LH) is inhibited by HGA in vitro in a linear, non-competitive way (K_i 120-180 mM), and that biosynthesis of hydroxylysine-derived intermolecular collagen cross-links was inhibited in a dose-dependent manner in organ culture (Murray et al., *J Clin Invest* 59:1071-1079, 1977). It was speculated that the results would explain the predilection of alkaptonuric complications for these tissues which contain collagens rich in hydroxylysine. Determination of urinary pyridinolines is a convenient and precise way to estimate LH activity in vivo (Steinmann et al. *Am J Hum Genet* 57:1505-8, 1995). We measured pyridinolines, which are the trifunctional, hydroxylysine-derived collagen cross-links, in urine from alkaptonuric individuals and controls. Urinary total lysyl pyridinoline (LP) and total lysyl pyridinoline (HP) were quantitated after acid hydrolysis by HPLC in three alkaptonuric individuals (age 24 and 35 years, asymptomatic; and 45 years, symptomatic). LP and HP expressed per creatinine and, more importantly, LP/HP ratios were normal (0.16-0.18; controls: 0.20 ± 0.05 , range 0.10-0.40), in contrast to those in 17 patients with Ehlers-Danlos syndrome type VI with documented LH deficiency (LP/HP = 6.0 ± 1.0 , range 4.3-8.1). Although we do not know precisely the relative contribution of the bone, cartilage and soft tissue compartments to the urinary pyridinoline pools, the results imply that significant inhibition of LH in vivo by HGA or its derivatives is unlikely. Thus, the pathogenesis of the disabling arthritic and life-threatening cardiovascular changes in alkaptonuric patients remains unexplained.

Very long chain fatty acid synthetase and fatty acid metabolism. *A.K. Heinzer¹, P. Watkins^{2, 1}, S. Kemp², K.D. Smith^{2, 1}.* 1) Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Kennedy Krieger Research Institute, Baltimore, MD.

Cells use fatty acids to make membrane lipids, signaling molecules, and energy. The metabolism of fatty acids is particularly important in mammalian brain where very long chain fatty acids (VLCFAs), fatty acids with carbon chains C22 or longer, are uniquely abundant in myelin. The importance of VLCFA homeostasis for normal brain function is highlighted by the severe neurologic abnormalities associated with VLCFA accumulation in patients with X-linked adrenoleukodystrophy (X-ALD) and peroxisomal biogenesis disorders, and *Drosophila* bubblegum dmBG mutants. To understand the metabolism of this important but little understood class of fatty acids, we have cloned and investigated genes encoding very long chain acyl-CoA synthetases (VLCS), enzymes that activate VLCFA to their CoA-esters. This is an essential first step because the CoA-ester is necessary for both anabolic and catabolic uses step because the CoA-ester is necessary for both anabolic and catabolic uses of VLCFAs. Using the published sequence of rat VLCS, we cloned a full length cDNA of mouse VLCS. We also cloned the mouse ortholog of dmBG, mBG, in silico. Expression of mVLCS or mBG in COS 1 cells produces a protein with VLCS activity. We have localized mVLCS to the peroxisome and the ER and mouse BG to the plasma membrane by transfecting COS1 cells with myc-tagged constructs. Northern blot and RT-PCR analyses indicate that mVLCS is primarily expressed in liver and kidney, with lower levels of expression in brain, heart, muscle and adrenal glands. mBG is expressed predominantly in brain and is down-regulated at the mRNA level in X-ALD fibroblasts. Although endogenous mVLCS cannot be detected mouse X-ALD fibroblasts, transfection of VLCS into these cells improves VLCFA β -oxidation. Since VLCS expression seems to affect the β -oxidation defect in X-ALD, we are screening patients for possible VLCS gene mutations that associate with X-ALD phenotype. A mouse VLCS knockout has been constructed to determine the significance of VLCS function in the intact organism and to investigate interactions with the X-ALD knockout mouse. bb.

Unnatural co-substrate-based rescue of enzyme activity in Refsum's disease resulting from mutations in R275 of phytanoyl-CoA hydroxylase. *M.D. Lloyd¹, M. Mukherji¹, N. Kershaw¹, C.H. MacKinnon¹, C.J. Schofield¹, A.S. Wierzbicki².* 1) Chemistry, University of Oxford, Oxford, United Kingdom; 2) Chemical Pathology, St. Thomas' Hospital, London, United Kingdom.

Phytanoyl-CoA hydroxylase (PAHX) is an iron(II), 2-oxoglutarate-dependent oxygenase involved in the metabolism of the isoprenoid fatty acid, phytanic acid. Mutations in PAHX cause Refsum's disease signs of which include retinitis pigmentosa, blindness and ataxia.

Highly purified recombinant PAHX was shown unambiguously to convert 2-oxoglutarate (2-OG) to succinate and carbon dioxide using phytanoyl-CoA as a substrate in vitro. The conditions for measurement of PAHX activity have been optimised and co-factor requirements clarified. The effects of mutations of arginine-275 (R275), the putative binding site for the 5-carboxylate of 2-OG, on activity were investigated. The inactive R275W and R275Q mutants present in some patients with Refsum's disease both retained activity <0.5nmol/min/mg when 2-OG is used as a co-substrate. Site-directed mutagenesis of similar sites in other 2-OG dependent oxygenases shows that they can have their activity restored by the addition of alternative 2-oxoacid co-substrates. A variety of 2-oxo-acids were assessed for their ability to 'rescue' the activity of these clinically identified R275 mutations and other possible R275 substitutions. 2-oxovalerate and 4-methylthio-2-oxo-butanoate rescued the activity of R275W PAHX to 7.19 and 5.52 nmol/min/mg, respectively, compared to a wild-type enzyme activity with 2-OG of 145nmol/min/mg. 2-oxobutanic acid rescued activity of R275Q PAHX at 8.39 nmol/min/mg. The R275A PAHX was rescued 7.06-10.12 nmol/min/mg by 4-methylthio-2-oxobutanoate, pyruvate, 2-oxobutanoate and 2-oxoisovalerate respectively. All three mutants were rescued to a lesser degree by other aliphatic 2-oxoacids.

These results imply that modified co-substrates can rescue enzyme activity in oxygenase disorders in vitro and merit investigation as possible therapies for inherited enzyme deficiencies where enzymes require co-factors or co-substrates for activity.

Identification and characterization of 15 novel PEX7 mutations which cause rhizomelic chondrodysplasia punctata (RCDP) and variant phenotypes. *P. Lin*¹, *C. Obie*¹, *G. Steel*¹, *A. Moser*², *H. Moser*², *N. Braverman*³. 1) HHMI, Baltimore, MD; 2) Kennedy Krieger Institute, Baltimore, MD; 3) Institute for Genetic Medicine, Johns Hopkins, Baltimore, MD.

RCDP is a peroxisome biogenesis disorder caused by mutations in PEX7, which encodes the receptor (Pex7p) for peroxisome matrix proteins containing a PTS2 targeting signal. We and others have previously reported 3 mutations in PEX7 which cause RCDP. We have now completed mutation analysis in 50 patients by direct sequencing of genomic DNA. We have identified a total of 18 unique alleles, accounting for 92 of 100 alleles. There are 14 missense (6 unique) mutations, 56 nonsense (7 unique, 50 are L292X, whose high frequency is due to a founder effect), 18 splice site (3 unique, 14 are IVS9+1G>C), 1 codon deletion and 1 codon insertion. Using polymorphic markers spanning the PEX7 gene, we show that IVS9+1G>C allele occurs on at least 3 different haplotypes and thus represents a hot spot for mutation. We performed ASO analysis for most mutations; none are population polymorphisms. To correlate genotype with phenotype, we divided the patients into 3 groups: those with severe plasmalogen deficiency and a classical RCDP phenotype (n=43), those with milder plasmalogen defects and milder phenotypes (n=5), and those with near normal plasmalogen levels and variant phenotypes (n=2). We performed Northern analysis to determine the levels of PEX7 mRNA and RT/PCR to evaluate the types of transcripts present in these patients. We expressed selected alleles in RCDP null cell lines and assayed the ability of mutant Pex7p to import PTS2 proteins. Our data show that both residual amounts of normal PEX7 mRNA and residual function of a mutant Pex7p can result in a milder phenotype.

Identification of a second locus for Refsum's disease at chromosome 6p22-24. A.S. Wierzbicki¹, M. Lambert-Hamill¹, J. Mitchell², M. Sidey³, M.D. Feher³, J. de Belleruche², F.B. Gibberd³. 1) Dept Chemical Pathology, St Thomas Hosp, London, England; 2) Dept Neuromuscular Disease, Charing Cross Hospital, London, England; 3) Dept Therapeutics, Chelsea & Westminster Hospital, London, England.

Refsum's disease is generally assumed to be caused by mutations in the peroxisomal enzyme phytanoyl-CoA hydroxylase (PAHX). It is characterised by adolescent onset progressive retinitis pigmentosa, anosmia, ataxia, deafness and sensory neuropathy with preservation of intellect. The sole enzyme abnormality in Refsum's disease is a deficiency in PAHX activity. We have previously shown that 55 per cent of patients with Refsum's disease in the UK do not show linkage to the PAHX locus on chromosome 10p13 in a study of 8 families comprising 48 living individuals, though they are phenotypically identical to patients with Refsum's disease linked to chromosome 10.

In the 4 families from the previous study not linked to chromosome 10 comprising 27 members with 9 affected individuals, further linkage analysis was conducted using a panel of 5 markers on chromosome 6p between D6S262 and D6S290. Linkage analysis indicated that a homogeneous cohort was present. A maximal LOD score of 1.72 was obtained in the region D6S314 to D6S308 corresponding to 6p22-24. Candidate genes in this region for Refsum's disease include the peroxin 7 (*Pex 7*): PTS-2 signal dependent transporter protein, mutations in which have previously been shown to cause a phenotypically distinct syndrome termed rhizomelic chondrodysplasia (RCDP). RCDP is characterised by rhizomelia, proximal bone shortening, disturbed endochondrial bone formation, intellectual retardation and multiple PTS-2 signal-containing peroxisomal enzyme deficiencies.

The data from this study suggests that the classical Refsum's disease phenotype, in common with other peroxisomal 'diseases', is actually caused by a genetically heterogeneous group of conditions.

Mammalian peroxisomal ABC transporters: functional characterization of the nucleotide binding fold (NBF) in health and disease. *P. Roerig, J. Grtner.* Pediatrics, University of Dsseldorf, Dsseldorf, Germany.

There are four ABC half transporter proteins in the mammalian peroxisomal membrane: adrenoleukodystrophy protein (ALDP), ALDP related protein (ALDR), 70 kDa protein (PMP70) and PMP70 related protein (P70R). Mutations in the ALD gene encoding ALDP result in the X-linked neurodegenerative disorder adrenoleukodystrophy (X-ALD). Clinical manifestations are highly variable with respect to age of onset, rate of progression and site of pathology. X-ALD is associated with elevated levels of very long chain fatty acids (VLCFA) and reduced VLCFA -oxidation. The interactions of peroxisomal ABC transporters, their functions in the peroxisomal import system and their role in fatty acid metabolism are poorly understood. Mutations in the ALD gene may impair these functions by affecting protein stability, ATP sites or substrate binding. To study the role of the NBF we generated wild type and mutant constructs of ALDP and PMP70. The mutant constructs included X-ALD patient missense mutations in the Walker A and 19mer region (G512S, S606L) and the corresponding PMP70 mutations (G478R, S572I). The NBF proteins were overexpressed in fusion with the maltose binding protein (MBP), purified by affinity chromatography and characterized by Western blotting. Photoaffinity labeling of the fusion proteins with 8-azido-ATP or 8-azido-GTP revealed a strong ATP and a negligible GTP binding. Using a coupled ATP regenerating enzyme assay for ATPase activity measurements we obtained the following kinetic parameters: Fusion protein V_{max} nmol/mol NBF/min km M ALDP-NBF-wt 64211,5 ALDP-NBF-G512S 27917,9 ALDP-NBF-S606L 66645,6 PMP70-NBF-wt 5818,2 PMP70-NBF-G478R 641161,8 PMP70-NBF-S572I 2989,9 The S606L and G478R mutants had a decreased ATP binding affinity while the G512S and S572I mutants decreased the maximum velocity of ATPase activity. We conclude that the NBF motifs of ALDP and PMP70 are important for peroxisomal ABC transporter function. They act as an ATP specific binding subunit releasing ADP after ATP hydrolysis. The NBF mutations and the consecutive ATPase dysfunction may contribute to the understanding of disease pathogenesis in these X-ALD patients.

Maternal metabolic enzyme genotype and risk of spontaneous abortions. *M. Romkes¹, C.S. Sims^{1,2}, R.D. Day¹, N. Markovic¹, T. Nukui¹, G. Harger¹, W.A. Hogge^{1,2}, R.B. Ness¹, W.L. Bigbee¹.* 1) University of Pittsburgh, Pittsburgh, PA; 2) Magee Womens Hospital, Pittsburgh, PA.

Conflicting reports concerning the relationship between maternal environmental/lifestyle factors, such as smoking or caffeine intake, and spontaneous abortions, may in part be attributable to genetic variation in metabolic activation and detoxification of these compounds. We examined the association between the risk of spontaneous abortion and metabolic enzyme genotype during an ongoing molecular epidemiological study evaluating the impact of tobacco smoke exposure during pregnancy. Information about pregnancy outcomes and various exposures was collected by means of a detailed interview questionnaire and smoking history was also evaluated by the determination of plasma cotinine levels. Blood samples from maternal subjects with a spontaneous abortion (n=49) and two independent sets of age, race and parity matched at-risk healthy women controls were genotyped for a panel of CYP and phase II enzymes (CYP1A1, GSTM1, GSTT1 and NAT2). The combination of a high activity CYP1A1 polymorphism and the NAT2 slow genotype was associated with a significantly higher risk of spontaneous abortions (odds ratio = 3.89; 95% CI 1.3 - 11.3, p=0.01). Neither tobacco exposure or alcohol consumption significantly contributed to risk. These preliminary data emphasize the need for future large scale studies to further assess the role of specific metabolic enzymes in gene-gene and gene-environment interactions with adverse outcomes in pregnancy which will likely yield new insights into the etiology, mechanism and risk of these events.

The Occurrence of Persistent Pulmonary Hypertension of the Newborn Correlates with Urea Cycle Intermediates and CPSI Function. *M.L. Summar¹, D. Pearson¹, S. Dawling², A. Bazyk³*. 1) Dept Pediatrics/Div Medical Genetics, Vanderbilt Univ Medical Ctr, Nashville, TN; 2) Dept. Pathology, Vanderbilt Univ Medical Ctr.; 3) Program in Human Genetics, Vanderbilt Univ. Medical Ctr.

Background: Limitation of endogenous NO production may play a role in the pathogenesis of persistent pulmonary hypertension in the sick term neonate. Endogenous NO is the product of the urea cycle intermediate arginine. Production of arginine depends on the rate-determining enzyme of the urea cycle, carbamyl phosphate synthetase (CPSI). Newborns possess less than half the normal urea cycle function making them particularly susceptible to minor changes in enzyme form and function. We hypothesized that newborns with PPHN would have lower NO and NO precursors and that a common functional exonic polymorphism (T1405N) in CPSI would correlate with the condition. **Methods:** Forty-seven neonates >2kg, >35weeks, and <72 hours old who were admitted to the Vanderbilt Neonatal Intensive Care Unit with (n=22) and without (n=25) echocardiographically-documented pulmonary hypertension were enrolled. Ammonia levels plasma, amino acid profiles and genotypes were obtained. **Results:** Patients who developed PPHN had an average arginine of 21.5umol/l while those who did not averaged 38.3umol/l (p=0.0004). The citrulline means were 6.1umol/l and 10.3umol/l respectively (p=0.02). Genotype analysis of PPHN patients for T1405N showed 5CCs, 17ACs, and 0AAs, whereas the controls had 7CCs, 16ACs, and 2AAs (Chi-square p=0.005 using the expected population allele frequency). Infants with the CC genotype had lower arginine and citrulline means (21.5umol/l and 5.8umol/l) than infants with the AA genotype (31.5umol/l and 13.5umol/l) consistent with a functional difference between the two forms of the enzyme. **Conclusions:** This study suggests that the development of PPHN in sick newborns is associated with inadequate availability of the urea cycle intermediates arginine and citrulline. The T1405N polymorphism in the CPSI DNA leads to diminished enzyme function and subsequent lower levels of NO precursors which may be critical in this vulnerable population.

Modification of gene expression in multiple carboxylase deficiency (MCD) by biotin treatment. *S. Solorzano-Vargas, D. Pacheco-Alvarez, A. Leon-Del-Rio.* Departamento de Biología Molecular, Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico. Mexico City.

Holocarboxylase synthetase (HCS) catalyses the biotinylation of three mitochondrial and two cytoplasmic carboxylases in humans. Inherited deficiency of HCS causes the disorder known as multiple carboxylase deficiency (MCD), characterized by metabolic ketoacidosis, abnormal urine organic metabolites and dermatitis. This disorder is potentially lethal if not treated promptly. . In almost all the cases, symptoms can be reverted by pharmacological doses of biotin. Although biotin is believed to be nontoxic in high doses, it has been shown recently that this vitamin affects the transcription of several genes which may be of relevance in the treatment of MCD.. We studied the effect of biotin deprivation and supplementation on the levels of mRNA of enzymes involved in biotin utilization by semiquantitative RT-PCR and northern blot analysis. Our results show that biotin upregulates the transcription of HCS and mitochondrial and cytoplasmic carboxylases. These results may explain the fast recovery of MCD patients once biotin supplementation is started.

Folding mutations in phenylketonuria: disease mechanisms explaining inconsistencies in the genotype-phenotype correlation. A. GAMEZ, B. PEREZ, R. VAZQUEZ, A. PEY, L.R. DESVIAT, M. UGARTE. CENTRO DE BIOLOGIA MOLECULAR, CSIC-UAM, MADRID, MADRID, SPAIN.

Phenylketonuria, an autosomal recessive genetic disease, is caused by mutations at the human PAH locus, which provoke an impaired function of the phenylalanine hydroxylase enzyme. We have used different experimental approaches in prokaryotic, eukaryotic and cell-free systems in order to explain the effect of different missense mutations, either lining (L348V and S349L) or outside the active site (I65T, R261Q, V388M and Y414C) as located in the PAH crystal structure (PDB accession code 1PHZ and 2PAH). All the mutations analyzed are structural mutations, except S349L, which is the only one affecting also the catalytic properties of the protein because it is involved in the structural maintenance of the iron binding site. The amount of mutant proteins and their residual activity can be modulated by lowering the growth temperature in COS cells and/or by GroES and GroEL co-overexpression in *E. coli*. In the prokaryotic system we analyzed the thermal inactivation profiles with and without chaperonins, the curves of the mutant enzymes are clearly shifted to lower temperatures demonstrating a reduced stability. This study was also confirmed by pulse-chase experiments using a coupled in vitro transcription-translation system. Size-exclusion chromatography shows altered oligomerization, partially corrected with chaperonins co-overexpression, except for the S349L mutant protein which is recovered as inactive aggregates. PAH subunit interaction is not affected in the I65T, L348V and V388M proteins as demonstrated in a mammalian two hybrid assay. The results suggest that interindividual cellular factors can modulate the severity of certain folding mutations, explaining the inconsistencies in the genotype-phenotype correlation.

Switching of substrate specificity between human and rat short/branched chain acyl-CoA dehydrogenases by in vitro mutagenesis. *M. He, J. Vockey.* Medical Genetics, Mayo Clinic, Rochester, MN.

The acyl-CoA dehydrogenases (ACDs) are a family of related enzymes which catalyze the α , β -dehydrogenation of acyl-coA esters, transferring electrons to electron transferring flavoprotein. We have previously cloned and expressed cDNAs for human and rat short/branched chain acyl-CoA dehydrogenase (SBCAD). Deficiency of this enzyme in humans has recently been described. The characterization of their substrate specificity suggests that the rat SBCAD is more active toward longer carbon side chains than human SBCAD, while the human enzyme can utilize substrates with longer primary carbon chains despite an overall amino acid homology of 89% between the two enzymes. To characterize the structural basis for these differences, a structural model for SBCAD was generated based on its homology to ACDs for which X-ray crystal data are available. The modeling identified 10 amino acids in or near the substrate binding pocket which differ in the rat and human enzymes. In vitro mutagenesis was used to systematically change these residues in the human enzyme to their rat counterpart in various combinations. Alteration of all 10 residues resulted in an enzyme with a substrate specificity that mimicked rat SBCAD. The combination of an A383T with either an L220M or L222M alteration was sufficient to significantly enhance the activity of human SBCAD towards substrates with longer carbon side chains, while a V104L/F105L mutation reduced human SBCAD activity towards longer primary carbon chain. Our data findings identify these amino acid residues as the primary determinants of the differing pattern of substrate utilization seen in SBCAD from rat and humans.

Functional Analysis of the Wilson Disease (WD) Copper Transporter. *G. Hsi¹, J.R. Forbes², M.M. Chen¹, D.W. Cox¹.* 1) Medical Genetics, University of Alberta, Edmonton, Alberta, Canada; 2) Biochemistry, McGill University, Montreal, Quebec, Canada.

WD is a copper transport disorder wherein patients accumulate copper due to impaired copper excretion via bile and have defective copper incorporation into the plasma ferroxidase ceruloplasmin. The gene, ATP7B, encodes a copper transporting P-type ATPase, ATP7B. The yeast orthologue of ATP7B is CCC2. In yeast, Ccc2p is required for high-affinity iron uptake. Ccc2p delivers copper to the plasma membrane oxidase Fet3p which, with Ftr1p, transports iron into the cell. In ccc2 mutants, Fet3p activity is absent and the mutant cannot grow on iron deficient media. Expression of ATP7B in ccc2 mutant cells functionally complements the phenotype. There are currently over 200 ATP7B mutations identified, of which 123 are missense (www.medgen.med.ualberta.ca/database.html). We used 2 approaches to discriminate disease mutations from rare normal variants: 1) expression in ccc2 yeast, which examines copper transport, and 2) expression in CHO cells, which examines intracellular localization. ATP7B is normally localized within the trans-Golgi network (TGN) under low copper conditions and redistributes to cytoplasmic vesicles under copper stimulation. We have generated 22 mutant constructs. Sixteen mutants have normal copper transport, 3 partially complement the phenotype and 2 are completely unable to complement ccc2 yeast. One construct resulted in no protein production when expressed in yeast. A class of mutants was identified which shows defective yeast copper transport and mislocalization to the endoplasmic reticulum (ER) in CHO cells. Of the mutants that show normal, or near normal, yeast copper transport, 2 classes are seen. One class shows partial mislocalization to the ER under low copper conditions and partial capacity to redistribute upon copper stimulation. The second class (1 mutant) did not redistribute in response to copper stimulation. Together, the yeast copper transport and the localization studies in CHO cells, provide important implications for patient diagnosis. Patients may have normal plasma ceruloplasmin but fail to excrete hepatic copper due to ATP7B mislocalization.

Exclusion of DFNB2 from 11q13.5 in 40 selected autosomal recessive non-syndromic deafness (RNSD) families.

L.M. Astuto, P.M Phil Kelley, J.W. Askew, W.J. Kimberling. Gene Marker Laboratory, Boys Town Hospital, Omaha, NE.

Deafness is the most common form of sensory impairment in humans. Mutations in several unconventional myosins, members of the large myosin superfamily of molecular motors that move along actin filaments, have been found to cause deafness in both humans and mice. Mutations in one human unconventional myosin, myosin VIIa (MYO7A), located at 11q13.5, have been reported to be responsible for both syndromic and nonsyndromic deafness. Usher syndrome is an autosomal recessive disorder characterized by hearing impairment and a progressive retinopathy. Usher syndrome type I, the most severe of the three clinical types, presents with congenital profound deafness, retinitis pigmentosa and vestibular areflexia. MYO7A mutations are responsible for Usher Ib, the most common genetic subtype of Usher I. Mutations in MYO7A are estimated to account for approximately 60% of all Usher I cases. Although, a wide spectrum of MYO7A mutations have been identified in Usher Ib patients, some mutations have been shown to cause DFNB2, a recessive deafness without retinal degeneration, and DFNA11, a dominantly inherited nonsyndromic hearing impairment as well as an atypical form of Usher syndrome suggesting that USH1B, DFNB2 and DFNA11 are MYO7A allelic mutants. Here, we describe the results of USH1B linkage analysis on 40 selected autosomal recessive nonsyndromic deafness families previously excluded by mutational analysis from DFNB2 (Cx26), the leading cause of nonsyndromic deafness. Both linkage and heterogeneity analyses of these families showed no evidence for DFNB2 within our sample. It is concluded that DFNB2 does not occur with any appreciable frequency in the USA population and that it may be limited to specific populations.

Further refinement of the Usher 1D locus at 10q21-22. X.Z. Liu^{1,2}, S.H Blanton¹, M. Bitner-Glindzicz³, A. Pandya¹, B. Landa¹, B. MacArdle⁴, K. Rajput⁴, T. Sirimanna⁴, B.T. Webb¹, R. Smith⁵, W.E. Nance^{1,2}. 1) Dept Human Genetics, VCU, Medical Col Virginia, Richmond, VA; 2) Dept Otolaryngology, VCU, Medical Col Virginia, Richmond, VA; 3) Inst Child Health, London, UK; 4) Dept Cochlear Implant, Hosp for Children, London, UK; 5) Dept Otolaryngology, Univ of Iowa, Iowa City, IA.

Usher syndrome (USH) is a devastating autosomal recessive form of hearing impairment, in which there is associated retinitis pigmentosa (RP) leading to progressive loss of vision. USH can be divided into three subtypes based on the severity and progression of the three major clinical findings. These syndromes are genetically heterogeneous, with at least six loci for USH1, three for USH2, and one for USH3. In the present study, five unrelated consanguineous families with USH1 were analysed for linkage to markers flanking the six USH1 loci. Two of these, including one Pakistani and one Turkish family living in England, demonstrated linkage to the USH1D locus. Another family had data consistent with linkage to USH1C. The remaining were not linked to any of the six USH1 loci, thus providing support for the existence of additional USH1 loci. The USH1D locus on 10q was originally mapped with a single consanguineous Pakistani family to a 15 cM interval on 10q21-22 with telomeric and centromeric markers at D10S529 and D10S573, respectively. Analysis of our two new USH1D families has allowed us to narrow the USH1D candidate region to a 7.3 cM interval with a telomeric flanking marker at D10S1752. Comparison of the affected haplotypes in our Pakistani family with the original Pakistani USH1D family yielded no evidence for a founder effect for either family. The identification of two additional affected families suggests that the USH1D may be a more common form of USH1 than originally suspected. Moreover, the refinement of the USH1D locus to a 7.3 cM interval should facilitate the positional cloning of the USH1D gene. (Work supported by the Deafness Research Foundation grant and by NIH grants DC04530, DC02530) NIDCD.

Genetic map localization of DFNA34 and DFNA36, two autosomal dominant nonsyndromic deafness loci. K.

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Hereditary deafness is genetically heterogeneous, and it is estimated that hundreds of genes may cause nonsyndromic sensorineural deafness. The identification of these genes associated with hearing loss continues to reveal the molecular mechanisms underlying the evolution, development, structure, and physiology of the auditory system, as well as the pathogenesis of hereditary hearing loss. We have ascertained two families, LMG113 and LMG128, segregating autosomal dominant, progressive, postlingual, nonsyndromic sensorineural hearing loss. The onset of the auditory phenotype in LMG128 occurs during the first decade of life and rapidly progresses to profound deafness by early adulthood, whereas LMG113 segregates a less severe phenotype that becomes clinically detectable during the third or fourth decade of life and progresses at a much slower rate. Genotype analysis excluded linkage to known dominant deafness loci in both families. A genome-wide linkage scan with STR markers revealed linkage of the deafness phenotype in LMG113 to a 14-cM region between markers D1S102 and D1S3739 on chromosome 1q44 (max. LOD = 3.33 at $q = 0$ for D1S2836). The obligate interval of this locus, designated DFNA34, overlaps that of the locus for Muckle-Wells syndrome (MWS), a dominant disorder characterized by periodic systemic inflammatory episodes and progressive sensorineural hearing loss. The overlap in phenotype and genetic locations suggest that DFNA34 and MWS may be allelic. Sensorineural hearing loss in LMG128 was linked to a 12-cM region between markers D9S1118 and D9S175 on chromosome 9q13-q21 (max. LOD = 5.51 at $q = 0$ for D9S1876). This locus was designated DFNA36, and its critical interval overlaps those for the nonsyndromic recessive deafness loci DFNB7/11, suggesting that these loci may represent allelic disorders. Linkage analysis of additional families such as LMG113 and LMG128 will contribute to the identification of the genes for deafness and will give insight into the pathophysiology and normal biological functions of these genes in the auditory system.

A novel locus DFNA 26 maps to chromosome 17q25 in two unrelated families with progressive autosomal dominant hearing loss. *T. Yang, R. Smith.* Genetics PhD Program, The University of Iowa, Iowa City, IA.

It is estimated that 15-25% of hereditary hearing loss has an autosomal dominant mode of inheritance. To date, 31 autosomal dominant loci have been mapped, and 11 of the associated genes, with diverse functions, have been cloned. We have mapped a novel locus for autosomal dominant non-syndromic hearing loss, DFNA26, to chromosome 17q25 in two unrelated American families with progressive autosomal dominant hearing loss. Two-point linkage analysis generates a maximum lod score of 3.20 and 5.06 for each family. Because the genetic map of this region is not completely consistent, we have used radiation hybrid mapping to establish marker order. We also have refined the candidate DFNA26 gene region by identifying new short tandem repeat polymorphisms (STRPs) and single nucleotide polymorphisms (SNPs). The former were identified by pulling out sequenced BACs using mapped ESTs; the latter were identified by SSCP analysis of STSs. Numerous genes are known to map to the region, and among the positional candidate genes, those functionally related to hearing are being screened for deafness-causing mutations.

Single nucleotide polymorphism discovery by the Cancer Genome Anatomy Project. *R. Clifford¹, M. Edmonson¹, T. Scherpbier¹, Y. Hu¹, R. Macdonald², P. Yip², A. Braun², K. Buetow¹.* 1) National Cancer Institute, Bethesda, MD; 2) Sequenom Inc., San Diego, CA.

Single nucleotide polymorphisms (SNPs) are the most common DNA variant in humans. These molecular markers can serve as reagents for constructing high-density genetic maps and performing gene based association studies. The Cancer Genome Anatomy Project Genetic Annotation Initiative (CGAP-GAI) of the National Cancer Institute seeks to identify genetic variations influencing susceptibility to cancer. To this end we employ informatics tools to predict SNPs from publicly available EST sequencing traces and in vitro techniques to verify the existence of candidate polymorphisms. To date we have identified 23,274 high quality candidate SNPs which lie in 6,174 UniGene clusters. 3,194 of these UniGenes correspond to named genes. We have validated more than 3,700 SNPs by sequencing, RFLP analysis, or mass spectrography, and we have confirmed that nearly 300 of the polymorphisms segregate as mendelian characters in CEPH families. Information about these molecular markers is available at <http://cgap.nci.nih.gov/GAI>. The CGAP-GAI website includes a number of tools for displaying information about SNPs and the genes in which they lie. Users can search for SNPs in a gene of interest by locus name, keyword, or GenBank sequence accession number. SNPs may also be retrieved by genetic or physical map location. Our website includes graphical display tools which show SNPs in the context of EST assemblies and the distribution of SNPs on genetic and physical maps. We are currently developing web based interfaces for showing the location of SNPs on the genome draft sequence and displaying SNPs relative to the predicted open reading frames of EST assemblies.

Refinement of the genetic and physical map of a primary ciliary dyskinesia locus *CILD2* on chromosome 19q13.4.

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Primary ciliary dyskinesia (PCD) is an autosomal recessive disorder with an incidence of 1:20000 live births characterised by sinusitis, recurrent pulmonary infection, bronchiectasis and subfertility. Approximately 50% of patients also have laterality defects, most commonly *situs inversus* (Kartagener syndrome). We have previously mapped a locus for PCD (*CILD2*) to chromosome 19q13.4 using homozygosity mapping and linkage analysis in five Arabic families, obtaining a multipoint lod score (HLOD) of 4.4 at $a = 0.7$ (M. Meeks, *et. al. J Med Genet* 2000; **37**:241-244). Identification and typing of novel microsatellites available from sequence data in the region has refined the candidate interval at the telomeric end to between markers *D19S572* and a novel microsatellite, designated *UCL288*, within cosmid R28830 (AC003682). This region corresponds to a physical distance of approximately 4 Mb, which is available in working draft or finished sequence (http://www-bio.llnl.gov/genome/html/chrom_map.html). Ciliary structural proteins, such as dyneins, are ideal candidate genes for PCD (Pennarun *et. al.. Am J Hum Genet* 1999; **65**:1508-1519). The region of interest is rich in kruppel-like zinc finger genes and we are using chromosome 19-specific databases and gene prediction programs to compile a complete transcript map. Homology searches using conserved motifs have so far failed to identify any dynein proteins in this candidate interval. Isolation of *CILD2* may identify a novel class of gene involved in ciliary structure/function.

Improving the quality of genotyping data. *J.C. Papp*^{1, 3}, *G. Kearsey*⁴, *K. Lange*^{1,2}. 1) Dept of Human Genetics and; 2) Dept of Biomathematics, UCLA, Los Angeles, CA; 3) Centre National de Génotypage, France; 4) Dataware Warwick, UK.

In the rush to process large numbers of genotypes to keep pace with modern automated laboratory methods, mistyping is likely to occur. Seemingly small genotype error rates can have a large impact on the prospect of obtaining positive linkage scores. Studies in which samples cannot be checked for simple Mendelian errors, such as sib-pair or case/control studies, are especially vulnerable.

We have developed arithmetical and statistical methods that can trap many of these errors and allow a more accurate data set to be passed on for further statistical analysis. The quality checks are both local and global. That is, each genotype is evaluated independently according to a number of quality parameters, then the overall data set is judged by population-based statistical methods. The tests are run from within a relational database into which all genotyping data is entered. The use of a relational database confers the additional advantages of improved integrity, management, manipulation, and presentation of the considerable amounts of data generated in large genome studies. Tests can be applied during the course of a study, as more data becomes available. Thus the results obtained are not only useful to correct errors contained in the genotype data, but can also be used as feedback to improve and streamline genotype interpretation and experimental protocols, and in making broader research decisions on experimental strategy and design.

To test our methods we evaluated at three stages of the quality control process the accuracy of real data from a genome-wide scan. The evaluation was performed using the mistyping analysis option of Mendel version 4, which detects Mendelian inconsistencies and unlikely double recombinants. The posterior error probability output by Mendel incorporates data on multiple markers and related family members. Mendel also permits the maximum likelihood estimation of genotyping error rates from pedigree data. Combining these analytic advances with the tests described above dramatically improves the quality enhancement process.

Melting Curve SNP (McSNP) Genotyping: A Simple Gel-Free Low Cost Approach to SNP Genotyping and DNA Fragment Analysis. *M.D. Shriver¹, J.M. Akey², D. Sosnoski¹, E. Parra¹, S. Dios¹, K. Hiester¹, B. Su², C. Bonilla¹, L. Jin².* 1) Dept of Anthropology, Penn State Univ; 2) Human Genetics Center, Univ. of Texas at Houston-HSC.

There is a clear need for improved single nucleotide polymorphism (SNP) genotyping methods. High-throughput applications require SNP genotyping methods that are gel-free, robust, inexpensive, and simple to perform. Although a number of high-throughput SNP methods have been developed, including the oligonucleotide ligation assay, minisequencing, a.k.a. genetic bit analysis, TaqMan, and dynamic allele specific hybridization, the requirements of simplicity and low-cost have not been adequately met. We have recently developed a method that is less expensive (\$0.25-0.50/genotype reagent cost including PCR) and easier to use than existing high-throughput genotyping assays. Two important steps in assaying DNA variation for high-throughput applications are discrimination of the variation and detection of the signal. We describe a novel SNP genotyping method called Melting curve SNP (McSNP) genotyping. McSNP combines a classical approach for discriminating alleles, restriction enzyme digestion, with a newer method for detecting DNA fragments, melting curve analysis. Melting is performed by heating DNA fragments in the presence of the double stranded DNA (dsDNA) specific fluorescent dye SYBR green. As the particular fragment is heated through its melting temperature, fluorescence decreases due to denaturation of the dsDNA. We show that it is possible to determine the composition of simple mixtures of DNA fragments such as those that result from restriction enzyme digestions of short PCR products. Using currently available instrumentation 96 samples can be analyzed and automatically scored in under 15 minutes. Since it is possible to engineer an artificial restriction site through the use of mismatched primers, McSNP is applicable to almost any SNP and not just those with natural restriction sites. Our results clearly demonstrate that McSNP is a simple, fast, inexpensive, and accurate means of genotyping SNP variation. This research is supported by grants from NIH/NIDDK (DK53958) and NIH/NHGRI (HG02154).

High throughput SNP discovery in candidate genes. *A.M. Valdes, I. Barroso, C. Baynes, R. Cubberley, T. Cutts, J. Durham, J. Greenham, A. Elliot, K. Jones, J. Lightning, I. Loudon, G. Maslen, M. Palmer, J. Reed, H. Ring, P. Swarbrick, K. Thomas, D. Townley, A. Varley, A. Schafer.* Incyte Genomics, Cambridge, England.

We have developed high throughput SNP discovery focused on candidate genes. The SNPs found (using fSSCP) have been confirmed by sequencing, mapped to genes, coding consequences assessed and allele frequencies computed. To date, approximately 4900 SNPs have been discovered and verified in a scan covering ~2,300 kilobases. The current rate is 220 kilobases/month screened in 47 samples (17 Caucasian, 13 Asian, 10 African American, 7 Hispanics). We selected candidate genes based on co-expression analysis, electronic library subtraction, positional cloning information and homology searches drawing from both public domain and proprietary sources. Therapeutic areas explored have been cardiovascular disease (84 genes), type 2 diabetes (170 genes), osteoporosis (50 genes), obesity (81 genes) and drug metabolism, absorption and excretion (ADME; 283 genes). Over 1900 SNPs have been discovered in these therapeutic areas. We have built gene structures by electronically mapping a coding sequence to the corresponding genomic sequence. Of the total SNPs screened to date 48% are located in exons (23% result in amino acid changes, premature stop or frameshift mutations), 40% are intronic and 9% are located in 3' and 5' UTRs. We present rates of transition and transversions and discuss the value of these data for genetic association studies, linkage scans and clinical trials. Amino acid changes in ADME genes can strongly affect individual's response to drug, such as efficacy and safety. We have characterized ethnic variation for a subset of 77 genes (203 SNPs) and observed statistically significant allele frequencies between ethnic groups at 38 exonic SNPs in 28 genes (36% of genes). Of these, 18 SNPs resulted in amino acid changes and in 5 cases the amino acid variant occurred in only one ethnic group. We present genetic distances and possible functional implications of the amino acid changes and discuss the impact of these results in future studies of adverse events and drug metabolism in clinical trials.

MAPPING A LOCUS FOR FAMILIAL IDIOPATHIC SCOLIOSIS ON CHROMOSOME 17q. *L. Baghernajad Salehi¹, M. Mangino¹, S. De Serio², D. De Cicco², F. Capon¹, S. Semprini¹, G. Novelli¹, B. Dallapiccola^{3,4}.* 1) Biopathology, Tor Vergata University of Rome, Rome, Italy; 2) Salvatore Maugeri Foundation, IRCCS, Cassano Murge, Bari, Italy; 3) Department of Experimental Medicine, La Sapienza University of Rome, Rome, Italy; 4) CSS Mendel Institute, Rome, Italy.

Idiopathic Scoliosis (IS) is a common condition whose etiology is still poorly understood, even if epidemiological studies suggest that IS has a significant genetic component. We report here linkage analysis of a three-generation Italian pedigree including 11 individuals affected by IS. We adopted a candidate locus approach and analysed 3 regions containing 3 genes whose murine homologues had been implicated in spine development. In particular, we examined the 2q35, 2q14 and 17q chromosome regions containing PAX3, GLI2 and WTN3A genes respectively. Linkage results excluded the first two loci, but generated significant data for the 17q region. In fact, marker D17S799 yielded a Z_{\max} value of 3.01 at $q=0.00$. Analysis of flanking markers confirmed these data. Thus, our study highlights WNT3A as a very attractive candidate for familial IS. Work funded by Ministry of Health.

Fine Mapping of the Acheiropodia locus on 7q36. *M.W. Kilpatrick¹, P. Ianakiev¹, S. Toledo², J. Correa Neto³, E. Lemos Silveira⁴, P. Tsipouras¹.* 1) Pediatrics, UConn Health Center, Farmington, CT; 2) Laboratorio de Genetica Medica da Faculdade de Medicina, Universidade Sao Paulo, Brazil; 3) Sao Paulo, Brazil; 4) Porto Alegre, Brazil.

Acheiropodia is a unique condition presenting with absence of hands and feet and characterized by truncations of the humerus and tibia and absence of fibula. There appears to be little variability of expression and with the exception of two affected siblings in Puerto Rico the reported cases are of Brazilian origin. The incidence of acheiropodia in Brazil has been estimated to be approximately 1/250,000 births. This severely handicapping malformation appears to affect only the extremities with no other systemic manifestations reported. Acheiropodia is inherited as an autosomal recessive trait and the heterozygotes are phenotypically normal. The vast majority of affected individuals are the offspring of consanguineous matings. Recently a locus for acheiropodia was mapped on chromosome 7q36. To facilitate the identification of the gene responsible for this disorder, we refined the acheiropodia critical region by haplotype analysis of a panel of acheiropodia families. Genotyping of these families for a panel of 12 highly polymorphic 7q36 markers defined a region of homozygosity by descent from D7S550 to D7S559 in all affected individuals. This identified a critical region for the acheiropodia locus spanning 8.4 centimorgans from D7S2447 to D7S2423. Furthermore, all affected individuals shared an identical D7S2465-D7S559 haplotype suggesting that the most likely region for the acheiropodia locus can be further reduced to the 3.2 centimorgans between D7S550 and D7S2423. Given the high recombination rate in this telomeric region, the physical size of the region is likely to be small. A number of genes have been mapped to this region of 7q36, including EN2, GBX1, INSIG1, NOS3, SHH, PTPR2, MRJ and HLXB9. The sonic hedgehog (SHH), homeo domain containing engrailed-2 (EN2) and homeobox HB9 (HLXB9) genes further map within the narrower D7S550-D7S2423 region. In the light of this and also given their putative functions, these genes might be considered good candidate genes for acheiropodia.

Benign hereditary chorea maps to chromosome 14q and evidence for genetic heterogeneity. *G.J Breedveld¹, L. Srinidhi², B.B.A. de Vries¹, A.K. Percy³, L.S Dure⁴, E. Ippel⁵, M.E. MacDonald², P. Heutink¹.* 1) Dept. Clinical Genetics, Erasmus University, Rotterdam, Netherlands; 2) Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown, USA; 3) Division of Pediatric Neurology, University of Alabama at Birmingham, USA; 4) Dept. Pediatrics, University of Alabama at Birmingham, USA; 5) Dept. Clinical Genetics, Academic Hospital Utrecht, Netherlands.

Benign hereditary chorea (BHC) is an autosomal dominant disorder characterized by early-onset nonprogressive chorea. The early onset and the benign course distinguishes BHC from the more common Huntingtons chorea. Using a large Dutch kindred we obtained strong evidence for linkage to markers on chromosome 14q (maximum lod score 6.32). Extensive haplotype analysis resulted in a critical region of approximately 12cM. A region that contains several strong candidate genes which are currently being tested. Four additional families with BHC were tested for relevant markers on chromosome 14. Two families showed evidence for linkage to the same region on chromosome 14 but two other families were not linked to this locus. Indicating genetic heterogeneity. These unlinked families will be used to search for additional loci. A detailed comparison between linked and unlinked families of clinical data will be presented.

Benign hereditary chorea with suggestive linkage to 14q: confirmation of a diagnostic entity and narrowing the critical region. *M.J. Fernandez^{1,3}, W.H. Raskind¹, J. Wolff^{1,4}, M. Matsushita¹, H. Lipe⁴, T.D. Bird^{1,2,4}.* 1) Departments of Medicine; 2) Neurology and; 3) Psychiatry, University of Washington School of Medicine, Seattle, WA; 4) Geriatric Research Service, Veterans Affairs Puget Sound Health Care System, Seattle, WA.

Benign hereditary chorea (BHC) or essential chorea differs from Huntington disease (HD) in several ways. It has early childhood onset, is not associated with other neurologic signs, and is non-dementing. Its severity peaks in the second decade and may even improve in old age. Although BHC appears to be transmitted as an autosomal dominant disorder, there has been some controversy as to whether non-HD familial chorea is a distinct disease or represents a heterogeneous group of disorders, as has been suggested by Schrag et al. (2000). Recently, however, linkage of BHC to a 20.6 KcM region on chromosome 14q was reported in a single large Dutch kindred (de Vries et al. 2000). This region was noted to contain several candidate genes of interest, including glial maturation factor beta, GTP cyclohydrolase 1, and the SMN interacting protein-1. Here we report a four generation family with nine affected members (five females and four males). We obtained samples from six affected individuals and one unaffected individual. The family was genotyped for 15 markers in a 23 KcM region on chromosome 14q between markers D14S49 and D14S66 that contains the putative BHC locus. Haplotype analysis supported linkage of disease in this family to this region of chromosome 14, providing further evidence of the existence of BHC as a distinct diagnostic entity. A recombination event that occurred in one affected individual enabled the critical region to be narrowed to a 6.93 KcM region between markers D14S1068 and D14S1064. Glial maturation factor beta and GTP cyclohydrolase 1 reside in this critical region, but SMN interacting protein-1 is excluded as a candidate gene as it lies outside the critical region. Identification of deleterious mutations would provide proof that BHC is a single gene disorder and mutation analysis is underway in this family.

Factor-analysis of Behavioral Characteristics in Autistic Disorder (AD). *M. Cuccaro¹, M.P. Bass², R.K.*

Abramson¹, S.A. Ravan¹, H.H. Wright¹, C.M. Wolpert², S.L. Donnelly², M.A. Pericak-Vance². 1) Dept Neuropsychiatry, W S Hall Psychiatric Inst, Columbia, SC; 2) Medicine, Duke University Medical Center, Durham, NC.

Autistic Disorder (AD) is a complex neurodevelopmental disorder with core impairments in social, communicative, and behavioral functioning. The AD phenotype has been well-characterized in terms of essential diagnostic features using the Autism Diagnostic Interview-Revised Edition (ADI-R). AD is a complex genetic disorder. Refinement of the phenotype in order to better understand homogeneous clinical subgroups may be useful in dissecting underlying AD genetic etiology. Thus, we conducted an exploratory factor analysis of our data set using 13 ADI-R items selected by the authors that are believed to represent repetitive interests and behavior. Resulting factor scores could then be used as traits for genetic analysis. We hypothesized that three factors would be identified reflecting sensory phenomena, repetitive interests and behavior, and insistence on sameness. Analysis of N=117 ADI-R protocols yielded two factors. The first factor consisted of five items related to repetitive behaviors and interests (ADI-R items: 72, 76, 77, 81, 84). The second factor consisted of three items related to insistence on sameness (ADI-R items: 73, 74, 75). Five of the items failed to sufficiently correlate with either factor (ADI-R items: 36, 70, 71, 78, 85). These findings suggest that empirically derived factors may be of value in characterizing the AD data sets for analysis of linkage and association.

No Evidence for Linkage of Familial Chiari Type 1 Malformation with the Williams Syndrome Locus on Chromosome 7. *D. Enterline¹, T. George¹, L. Osborne², B. Pober³, A. Franklin¹, C. Wolpert¹, E. Bonner¹, S. Slifer¹, T. Milhorat⁴, M. Speer¹.* 1) Radiology, Duke University Medical Center, Durham, NC; 2) University of Toronto, Toronto, Ontario; 3) Yale University School of Medicine, New Haven, CT; 4) SUNY Brooklyn, NY.

Chiari type 1 malformation (CM1) is a rare condition with a prevalence of $\sim 1/5000$, defined traditionally as ≥ 5 mm tonsillar descent through the foramen magnum, and more recently by a volumetrically "too small posterior fossa." Two lines of evidence suggest a genetic component to CM1: (i) familial aggregation has been established and (ii) CM1 is associated with a variety of known genetic conditions including Williams-Beuren Syndrome (WBS). WBS is a developmental disorder characterized by the deletion of 17 genes on chromosome 7q, including elastin. We tested the markers D7S1870, D7S613, and D7S489 (3 distinct copies) which span the 2 cM deletion interval flanking the elastin gene in a series of 10 multiplex families in which two or more members have a confirmed diagnosis of CM1. Assuming an autosomal dominant "affecteds only" analysis, all two-point lod scores were negative. Multipoint lod scores were also negative, excluding the WBS region at the -2.0 lod score criterion. Three of 10 pedigrees demonstrate haplotypic exclusion of this region. These data suggest that genes within the WBS deletion region are unlikely to be involved in the development of CM1 in this series of pedigrees.

Respective frequencies of ARCMT loci in 45 consanguineous families. Toward the identification of the gene localized in 5q32. *A. Guilbot¹, C. Verny¹, C. Bachelin², A. Gabrels-Festen³, A. Bouhouche⁴, D. Grid⁵, A. Baron², A. Brice¹, E. LeGuern¹.* 1) INSERM U289, Hopital de la Salpetriere, Paris, Paris, France; 2) CJF 9711, Faculte de Medecine, Hopital de la Pitie-Salpetriere, Paris, France; 3) Institute of Neurology, University Hospital Nijmegen, The Netherlands; 4) Laboratoire de Neurogenetique, Hopital des specialites, Rabat, Morocco; 5) Genethon, Evry, France.

Charcot-Marie-Tooth (CMT) disease is a heterogeneous group of disorders that affect the peripheral nervous system (PNS). CMT is usually inherited as an autosomal dominant disorder, although recessively inherited forms were described. Six loci are now characterized as responsible for the autosomal recessive forms of demyelinating CMT (ARCMT) from which two genes were recently identified. In this study, 45 consanguineous families with ARCMT of various origin were listed for linkage to the 6 known loci. Homozygosity mapping supports that in 1/3 of them, the ARCMT was linked to one of the known region. We found five consanguineous families with ARCMT linked to the 5q32 region. Moreover, 5 Dutch and 1 Turkish families were reported to be 5q-linked. All these families were genotyped for 8 new polymorphisms which led us to identify a common region segregating with the disease. Two different disease haplotypes were determined according to the ethnic origin. A combined YACs/BACs contig spanning 2 Mb of the 5q32 region of interest was used to map ESTs within this interval. The PNS expression patterns of the candidate sequences were determined in order to identify genes expressed in Schwann cells or in peripheral nerves. Further investigations are in process for two candidates.

Progress in a genome scan for anxiety disorders. *J. Gelernter^{1,2}, K. Bonvicini¹, G. Page³, S.W. Woods², A. Goddard², S. Kruger^{1,2}, D.L. Pauls⁴, S. Goodson¹.* 1) Psychiatry 116A2, VA CT Healthcare System, West Haven, CT; 2) Dept. Psychiatry, Yale Univ. School of Medicine, New Haven, CT; 3) Dept. Biometry and Epidemiology, MUSC, Charleston, SC; 4) Child Study Center, Yale Univ. School of Medicine, New Haven, CT.

Several anxiety disorders are heritable, but no confirmed linkages for any have been identified to date. We are conducting a genome scan in a set of 19 American pedigrees (average size 9.3 individuals, total 176 subjects) segregating panic disorder, agoraphobia, social phobia, and simple phobia, with the goal of locating susceptibility loci. Diagnoses were made on the basis of semi-structured SADS interview. With data from 206 microsatellite loci, several promising regions with potential linkage to anxiety disorders have been identified. The most promising possible linkage, to the agoraphobia phenotype, is on chromosome 3, and is supported by a multipoint NPL score (computed with GENEHUNTER) of 3.01 ($p=0.0034$, calculated empirically), with most of the evidence for linkage contributed by a single family. A SIMLINK analysis with 100 replicates showed that this set of pedigrees is expected to generate average lod scores of 7.8 and 10.6 under dominant and recessive models, respectively (under genetic homogeneity). We are currently genotyping additional markers to complete a genome scan at 10 cM density. SUPPORT: VA, NIMH.

Childhood Absence Epilepsy in 8q24: Refinement of Candidate Region and Construction of Physical Map. Y.

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Childhood absence epilepsy (CAE), one of the common idiopathic generalized epilepsies, accounts for 8% to 15% of all childhood epilepsies. Inherited as an autosomal dominant trait, frequent absence attacks start in early or mid-childhood and disappear by 30 years of age or may persist through life. In 1998, we mapped the locus for CAE persisting with tonic-clonic seizures to chromosome 8q24 (ECA1; MIM No. 600131) by genetic linkage analysis. As a further step for the identification of *ECA1* gene, we constructed a BAC and YAC-based physical map for the 8q24 region. This map consists of 23 BAC clones and four YAC clones, and which spans about 3Mb between D8S1710 and D8S523 including 37 STS markers. Detailed haplotype analysis with correct order of STS markers according to the physical map reduced the *ECA1* region to 1.5 Mb flanked by D8S554 and D8S502. Pairwise analysis in six families confirmed linkage with the pooled LOD score of 4.10 ($q = 0$) at D8S534. The sequence-ready physical map as well as the narrowed candidate region described here should contribute to the identification of the *ECA1* gene. Mutational analysis and characterization of genes on our physical map are on going looking for *ECA1* gene.

Clinical Characteristics and Linkage Analysis of One Chinese Benign Familial Infantile Convulsions Kindred.

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Benign familial infantile convulsions (BFIC) is a rare idiopathic epileptic syndrome transmitted as an autosomal dominant trait. In 1997, Guipponi mapped the gene for BFIC to chromosome 19q by linkage analysis. Recently, Giordano suggested a second locus was involved in this disease. Here we report one big Chinese BFIC kindred that was not linked to chromosome 19q. The proband was a 7 months old infant with generalized tonic-clonic seizure first at 3 months old, which had a family history of seizure with an onset at 3-8 months of age. Sleeping EEG in ictal period showed spike-slow waves in bilateral central parietal and temporal lobe areas. Cerebral MRI showed bilateral frontal lobes hypoplasia. He was treated with sodium valproate for 6 months interruptedly, the seizure frequency reduced apparently and EEG showed improved with normal psychomotor development. Analogous seizures were identified in 10 of 30 individuals at risk in this kindred. Their seizure onsets began at 3 to 8 months of age and disappeared spontaneously before 3 years old, normal psychomotor development, favorable outcome. Considering the kindred trees, it seemed clear that this disease was transmitted as an autosomal dominant trait. Purpose: To conduct linkage analysis between the kindred locus phenotype and chromosome 19 markers. Methods: Five microsatellite DNA markers (D19S49, D19S250, D19S414, D19S416, D19S245) were chosen to make haplotype and linkage analysis of this BFIC kindred. Results: This Chinese kindred was diagnosed as BFIC. Among the three markers D19S49, D19S416, D19S245, the maximum LODs was located on D19S416. When the recombinant rate was 0.3, the maximum LOD score was 0.52; when the recombinant rate was 0, the LOD score was -. When the recombinant rate was 0.1, the LOD score was less than 0. D19S414 and D19S250 could not provide information. Conclusions: This BFIC kindred locus phenotype was not linked to chromosome 19q, this suggests BFIC exist heterogeneity.

Genetic variation of serotonin system genes in a sample of autism families from Portugal. *A.M. Vicente¹, A. Coutinho¹, L. Mota-Vieira³, C. Marques², G. Oliveira².* 1) Instituto Gulbenkian Ciência, Oeiras, Portugal; 2) Hospital Pediátrico de Coimbra, Coimbra; 3) Hospital Divino Espírito Santo, Ponta Delgada, Azores.

Autism is behavioral syndrome characterized by deficiencies in social interaction and communication and by repetitive and stereotyped behaviors. Aiming at the identification of susceptibility factors in autism, we initiated the collection of a sample of nuclear and multiplex families with autism. Thus far 68 families, including 70 autistic patients and 45 relatives affected with other PDD's or cognitive/neuropsychiatric disorders, have been recruited from mainland Portugal and from the Azorian islands. Given its geographical isolation, this population has retained genetic homogeneity characteristics particularly valuable for this study.

Anomalies in the serotonin (5-HT) system have been implicated in a number of neuropsychiatric disorders, including autism. We have used the Transmission Disequilibrium Test to evaluate, in our sample, known polymorphisms in the 5-HT transporter (5-HTT) and the 5-HT_{2A}, 5-HT_{1D} and 5-HT_{2C} receptor genes. No evidence for association of the receptor's gene polymorphisms or the 5-HTTLPR promoter polymorphism was found in this sample ($p > 0.05$). However the polymorphism at the intron 2 of the 5-HTT gene yielded evidence for association ($p = 0.03$). This result contrasts with previous reports of linkage disequilibrium of the 5-HTTLPR but not the 5-HTT intron 2 marker with autism, and does not support a functional role for the 5-HTTLPR variants in our sample.

The results obtained in this relatively small sample require further investigation. In particular, given the high prevalence of cognitive and neuropsychiatric disorders observed in our families, and the reports of association of serotonin system genes in diverse behavioral traits, we are currently evaluating family members affected with neuropsychiatric disorder/cognitive deficiencies, under the hypothesis that heterogeneous expression may be mediated by the same underlying genetic susceptibility factors.

Testing for genetic associations of the PAX genes in a spina bifida population. *K.A. Volcik¹, S.H. Blanton², G.H. Tyerman³, H. Northrup¹.* 1) Dept of Pediatrics, University of Texas Houston Medical School, Houston, TX; 2) Dept of Pediatrics, University of Virginia, Charlottesville, VA; 3) Shriners Hospital for Children, Los Angeles, CA.

Neural tube defects (NTDs) are among the most common severely disabling birth defects in the US, affecting approximately 1-2 of every 1000 live births. The etiology of NTDs is believed to be multifactorial, involving the combined action of genetic and environmental factors. We have utilized a nonparametric method, the transmission disequilibrium test (TDT), to test candidate genes which could possibly play a role in the formation of NTDs. We have tested DNA from ~500 spina bifida (SB) affected individuals and their parents for linkage and association utilizing polymorphic markers from within or very close to the candidate genes of interest. The PAX genes encode transcription factors that play a role in embryonic development, including neural tube development. Mutations in PAX genes have been shown to be responsible for the phenotype of several mouse NTD models, including the *splotch* and *undulated* mouse models. TDT analysis produced positive results for PAX1 flanking marker d20s101 ($P=0.022$). Single strand conformational analysis has been performed on all SB patients and their parents for the paired domain region of PAX1. Four distinct band shifts have been detected in the paired domain region of PAX1: 1) one seen in a single SB patient and his father, but absent in his mother, 2) one seen in multiple individuals, including SB patients and parents, 3) one seen in a single SB patient and her mother, with information on her father unavailable, 4) one seen in three individual SB patients, two for which their mothers do not have the band shift and the fathers are unavailable, the third for which the mother has the shift and the father is unavailable. We are currently in the process of sequencing these band shifts in order to determine the role these variations may play in NTD development.

Novel psychometric analyses for use in genetic studies of behavioral and psychological traits. *S.H. Shaw*¹, *T. Chu*², *M.A. Schork*², *N.J. Schork*². 1) Psychiatry and Medicine, University of California, La Jolla, CA; 2) Genset Corp. LaJolla, CA.

The dissection of the genetic basis of personality, behavioral, and general psychological traits, such as depression, anxiety, and novelty-seeking, is often confounded by complexities associated with phenotypic definitions and underlying etiologic heterogeneity. Population screening tools for personality and behavior are often based on psychological scales whose components are frequently ignored in the final analyses. We have developed a number of analytic tools that can be used to more adequately and appropriately extract information from psychological scales in genetic linkage and association studies. Many of these tools have their origins in traditional multivariate statistical analysis methods. These tools can be used in large-scale linkage and association analyses, and we document power increases associated with their use in these settings using both actual and simulated data. We describe the derivation of our methods as well as the results of their application in a number of studies. We also discuss extensions of our methods and areas for further research.

Mutational and Linkage Analyses of Atrial Septal Defect. *Y.H. Ching¹, S.J. Cross¹, A. Gilliland-Paterson¹, A. Dearlove², M. Rhodes², R. Newbury-Ecob³, D. Bonnet⁴, J.D. Brook¹.* 1) Institute of Genetics, University of Nottingham, Queens Medical Centre, Nottingham, U.K; 2) HGMP-Resource Centre, Hinxton, Cambridge, U.K; 3) Clinical Genetics Service, Institute of Child Health, Bristol Royal Hospital for Sick Children, St. Michael's Hill, Bristol, U.K; 4) Service de Cardiologie Pédiatrique, Groupe-Hospitalier Necker, 139, Rue de Sevres, Paris, France.

Heart disorders represent the most common form of congenital abnormality in humans and of these atrial septal defects (ASDs) are amongst the most common. In a heart with an ASD there is a hole between right and left atria which causes mixing of oxygenated and deoxygenated blood.

Mutations in NKX2.5 have been found in several families with ASD and atrioventricular conduction defect. We have screened a panel of ASD families using SSCP analysis. We have identified a mutation which is a splice-site change in the 5' end of intron 1. This is the second report of a splice site mutation in NKX2.5 causing ASD with atrioventricular conduction defect.

A genome-wide linkage analysis has been carried out in French and British families which show dominantly inherited secundum atrial septal defects with no atrial ventricular conduction block. These families should provide a maximum LOD score of over 5. Over half the genome has been excluded with 95% confidence and the study is ongoing.

A Genome-wide Scan for CHD Susceptibility in the Saguenay-Lac-Saint-Jean region of Quebec. *J.C. Engert¹, M.-C. Vohl², P. Lepage¹, C. Dor¹, C. Brewer¹, D. Frappier¹, A. Verner¹, J. Platko⁵, J. Rioux⁵, D. Gaudet³, K. Morgan^{1,4}, T.J. Hudson^{1,4,5}.* 1) Montreal Genome Ctr, L3-401, Montreal General Hosp, Montreal, PQ, Canada Montreal Genome Centre, McGill University Health Centre, Montreal, Quebec, Canada; 2) Lipid Research Center, CHUL Research Center, Laval University, Sainte-foy, Quebec, Canada; 3) Hopital de Chicoutimi, Chicoutimi, Quebec, Canada; 4) Departments of Human Genetics and Medicine, McGill University, Montreal, Quebec, Canada; 5) Whitehead Institute for Biomedical Research, MIT, Cambridge, MA.

One of the leading causes of death in the western world is coronary heart disease (CHD). This disease has long been known to exhibit familial aggregation. While some cases of early-onset CHD are due to rare monogenic disorders such as familial hypercholesteremia, the majority of CHD is thought to have a multifactorial origin. The factors characterized to date fall into several pathogenic categories: hyperlipidemia, hypertension, diabetes and obesity. While these conditions account for a significant portion of CHD patients, there exist currently undefined contributors to the genetic risk for this disease. In order to identify novel genes, we examined families from the Saguenay-Lac St.-Jean (SLSJ) region of Quebec with early-onset CHD, that do not have hyperlipidemia, as the genes responsible for this intermediate phenotype have already been investigated in this population. To identify loci that contribute to CHD susceptibility, we performed an initial whole genome scan on 187 individuals from 20 families (an average of 6-7 sibs/family) from the SLSJ, a population known to exhibit a founder effect. By using 378 evenly spaced polymorphic microsatellite markers, a 9.2 cM resolution was achieved. Markers had an average heterozygosity of 73%. Multipoint nonparametric linkage analysis using the GENEHUNTER and GENEHUNTER-PLUS software packages identified four chromosomal regions with NPL scores greater than 1.8. These were located on chromosomes 7, 8, 9, and 12. We are currently fine mapping these four regions and characterizing candidate genes for their contribution to CHD susceptibility in this population.

Markers of lipoprotein lipase and angiotensin I converting enzyme are linked to young-onset hypertension in Taiwan. C.S. Fann¹, J.-W. Chen², Y.-S. Jou³, S.-Y. Wu^{1, 4}, W.-H. Pan¹. 1) Epidemiology and Public Health, Inst Biomed Sci, Acad Sinica, Taipei, Taiwan, Taiwan; 2) Department of Cardiology, Veteran General Hospital, Taipei, Taiwan; 3) Division of Molecular and Genomic Medicine, NHRI, Taipei, Taiwan; 4) Institute of Life Sciences, National Defense Center, Taipei, Taiwan.

A genetic linkage study of young-onset hypertension was performed using data from 59 nucleus families of Han Chinese residing in Taiwan. Thirty seven microsatellite markers near 18 hypertension candidate genes were genotyped. In a nonparametric IBD sib-pair analysis, a positive linkage signal was found for 4 microsatellite markers: D1s1612 ($p = 0.0162$), D1s547 ($p = 0.0263$), D8s1145 ($p = 0.0284$), and D17s2193 ($p = 0.0256$) which were located near genes of atrial natriuretic peptide / glucose transporter 5, angiotensinogen, lipoprotein lipase (LPL), and angiotensin I converting enzyme (ACE), respectively. Marker D5s1480 located near α_2 -adrenergic receptor had a borderline level of significance ($p = 0.0785$). Comprehensive genotyping with more markers in these regions was carried out to confirm whether these genes are linked to young-onset hypertension. Nonparametric linkage (NPL) analysis showed that LPL marker of the LPL gene was significantly linked to young-onset hypertension with a NPL score of 3.3. Transmission disequilibrium test showed that LPL and D8s332 markers of LPL gene and ATA108A05 marker of ACE gene were significantly ($p < 10^{-3}$) associated with young-onset hypertension. These results suggest that LPL and ACE may play causal roles in the development of young-onset hypertension.

Paraoxonase phenotype is a better predictor of vascular disease than PON1₁₉₂ or PON1₅₅ genotype. *G.P. Jarvik, L.S. Rozek, V.H. Brophy, T.S. Hatsukami, R.J. Richter, L.A. McKinstry, G.D. Schellenberg, C.E. Furlong.* The University of Washington Dept of Medicine (Medical Genetics), Seattle.

The paraoxonase (PON1) PON1-Q192R and PON1-L55M polymorphisms have been inconsistently associated with vascular disease. Plasma PON1 activity phenotypes vary markedly within genotypes, and were, therefore, expected to add to the informativeness of genotype for predicting vascular disease. Our case-control study included 212 age and race matched men with mean age 66.4 yr. (range 49-82 yr.); 95% were Caucasian. The 106 carotid artery disease (CAAD) cases had >80% carotid stenosis and the 106 controls had <15% stenosis by ultrasound. Both PON1 substrate hydrolysis rates (paraoxon, POase; diazoxon, DZOase) were significantly lower in cases than in controls and were significant predictors of CAAD using logistic regression (POase, $p=0.005$, 25% reduced; DZOase, $p=0.019$, 16% reduced). DZOase predicted vascular disease independently of lipoprotein profile, HDL subfractions, apoAI, and smoking. PON1-192 ($p=0.75$) and PON1-55 ($p=0.83$) genotypes or haplotype ($p=0.70$) did not predict case-control status unless the activity phenotype was also included as a predictor using logistic regression. When phenotype was included as a predictor both PON1-192 and PON1-55 genotypes or combined haplotypes were significant predictors of CAAD at the 0.05 level.

We have recently detected a novel, frequent (freq=0.2, n=66) PON1 promoter polymorphism that is associated with a 50% increase in POase and DZOase activity and is in linkage disequilibrium with PON1-55. We are currently evaluating whether this polymorphism confounds the relationship of PON1-192 or PON1-55 genotype with CAAD.

In conclusion, examining PON1-192 and/or PON1-55 genotypes alone may mistakenly lead to the conclusion that there is no role of PON1 in CAAD. This may have broad implications for the utility of the 'genotype only' approach. These results support the benefit of a 'level crossing' approach that includes intervening phenotypes in the study of complexly inherited disease.

Systematic survey of SNPs (single nucleotide polymorphisms) in the candidate responsible genes for myocardial infarction in the Japanese population. *Y. Ohnishi¹, T. Tanaka¹, R. Yamada¹, M. Hori², Y. Nakamura¹.* 1) Hum Genome Ctr, Inst Med Sci, Univ Tokyo, Minato-ku, Tokyo, Japan; 2) Dep of Internal Medicine and Therapeutics, Osaka Univ, Suita, Osaka, Japan.

Acute myocardial infarction (MI) is one of the cardiovascular diseases that is characterized by sudden occlusion of coronary arteries resulting in necrosis of cardiac muscle. Although the onset of MI is in some part related to life, it is also influenced by complex patterns of inheritance. To identify genes defining susceptibility to MI, an association study to include hundreds of thousands of SNPs throughout the genome is necessary. On the basis of this prospect, we just initiated genome-wide and systematic searches for SNPs in Japanese population. These SNPs will lead to an even denser map of SNPs. Prerequisite to the large scale study, we have surveyed 50 candidate responsible genes of MI for SNPs by directly sequencing 96 independent alleles derived from 48 unrelated Japanese patients with MI. Surveyed regions included 29.2 kb of 5' flanking regions, 67.1 kb exonic and 40.9 kb intronic sequences, and 1.8 kb of 3' flanking regions. In total, we identified 219 SNPs: 60 in 5' flanking regions, seven in 5' untranslated regions (UTRs), 62 in coding elements, 73 in introns, 16 in 3' UTRs, and one in a 3' flanking region. Among the 62 coding SNPs, 27 were non-synonymous changes. Allelic frequencies of some of the polymorphisms were found to be significantly different from those reported in European populations. For example, Q506R substitution in the coagulation factor V gene, so-called "Leiden mutation", has a reported frequency of 2.3% in Europeans. However, we did not detect the Leiden mutation in any Japanese we investigated. This data support the hypothesis that some SNPs are specific to particular ethnic groups. Our ongoing large-scale SNP survey will enable us to identify novel MI susceptibility genes through association studies.

Mutation screening of candidate genes in the critical region of FDCM mapped to chromosome 1q32. *D. Li, A. Karibe, G. Czernuszewicz, O. Gonzalez, L. Bachinski, R. Roberts.* Dept Medicine/Cardiology, Baylor Col Medicine, Houston, TX.

Familial idiopathic dilated cardiomyopathy (FDCM, OMIM 601494) is a genetically and clinically heterogeneous group of primary cardiac muscle disease that accounts for at least 30 percent of the cases of idiopathic dilated cardiomyopathy and is a major cause of congestive heart failure necessitating heart transplantation. FDCM patients often present with ventricular chamber enlargement, systolic dysfunction, heart failure and sudden death. Using a candidate gene approach, mutations of cardiac actin and desmin were identified as genetic causes of FDCM in a few small families. Out of 9 chromosomal loci mapped for autosomal dominant FDCM, only one gene, namely lamin A/C, has been recently identified. We previously identified a large family with FDCM inherited as an autosomal dominant trait and mapped the disease gene to chromosome 1q32 by linkage analysis. The disease gene-bearing region was subsequently defined by extensive haplotype analysis to be between D1S2840 and D1S249. Because the large size of the critical region (8.6cM) precluded positional cloning, our efforts to identify the FDCM gene at 1q32 have been focused on candidate genes. Candidate genes have been selected and evaluated on either pathological, expression, or genetic grounds. These include (in part): hPMCA4 (a plasma membrane calcium ATPase), fibromodulin, renin, myosin-binding protein-H, cardiac troponin-T, transforming growth factor beta, the muscarinic cholinergic receptor, the lamin B receptor, transcription factor SL1, and PRELP. Either cDNAs or exons of these genes were amplified from the patients and control subjects with RT-PCR or PCR and were subjected to sequencing. No mutations were detected in the coding regions of any of these candidate genes we sequenced. In order to identify candidate genes responsible for the family, we have obtained EST clones of cardiac origin claimed to map to the region of 1q32. DNA made from these EST clones were blotted onto a dot blot and probed with heart cDNA. The hybridization results demonstrated that twelve clones are highly or moderately expressed in the heart. These genes will be further evaluated and screened for the mutation.

Evidence for linkage to 13q21.1-q33.1 in a family with dilated cardiomyopathy and conduction system disease.

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Familial dilated cardiomyopathy (FDC) accounts for at least 25% of cases of idiopathic dilated cardiomyopathy, and causes heart failure and conduction system disease. Several FDC loci (1p1-1q1, 1q32, 2q11-q22, 2q31, 3p22-p25, 6q23, 9q13-q25, 10q21-23) and three disease genes - cardiac actin (15q14), lamin A/C (1q21.1-q21.2) and desmin (2q35) - have been identified. However, the genetic etiologies of most cases of FDC remain unknown. A four generation Caucasian kindred with autosomal dominant transmission of FDC was clinically evaluated. Nine surviving and two deceased individuals in this family were characterized with conduction system disease, less prominent systolic dysfunction and mild ventricular dilatation. We successfully adopted a method of DNA pooling and shared segment analysis of an autosomal dominant trait to streamline our genome scan and subsequently identified a new FDC locus. The critical region is between markers D13S1320 and D13S173 (13q21.1-q33.1), with a maximum multipoint LOD score of 3.20 at D13S1230 ($\theta = 0.0$). The identification of another locus for FDC emphasizes its genetic heterogeneity and may assist in the elucidation of the causes of this disease. We have yet to identify potentially interesting candidate genes in this large, 40cM chromosomal region, and our first approach will focus on the identification of other families with a similar phenotype and linkage to chromosome 13.

Analysis of the angiotensinogen gene in the MRC British Genetics of Hypertension Study. *P.B. Munroe¹, J. Knight¹, W. Lee², N. Brain², J. Pembroke¹, M. Brown³, N. Samani⁴, N. Benjamin¹, M. Farrall (i)⁵, D. Clayton³, P. Ratcliffe (i)⁵, J. Webster (ii)⁵, M. Lathrop (iii)⁵, A. Dominiczak², J. Connell², M. Caulfield¹.* 1) St Bartholomew's and The London Medical School, London, UK; 2) University of Glasgow, UK; 3) University of Cambridge, UK; 4) University of Leicester, UK; 5) University of Oxford, UK (i), University of Aberdeen, UK (ii), CNG, Paris (iii).

The MRC-funded British Genetics of Hypertension (MRC BRIGHT) study is a national collaboration funded to identify 1500 hypertensive families, based upon affected sibling pairs, with the primary goal of undertaking a genome wide screen for blood pressure genes. The angiotensinogen gene (AGT) has emerged as a strong candidate for essential hypertension in recent years and both positive association and linkage of AGT markers with hypertension have been reported. However not all the genetic studies have been positive, and the MRC BRIGHT study is an attractive resource in which powerful candidate gene studies can be performed. In this study we assessed whether AGT is linked to hypertension in affected sibling pairs from the 502 families from the BRIGHT resource. A polymorphic microsatellite marker located ~500Kb from the AGT locus was genotyped in all hypertensive families using the polymerase chain reaction with allele separation on 5% 96-lane acrylamide gels on a 377 sequencing machine. The computer program SPLINK was used to perform linkage analysis, allele frequencies were calculated from the probands in each family. No evidence of linkage was found between the AGT locus and essential hypertension ($p = 0.583$). This suggests that AGT is not linked to hypertension in the BRIGHT population studied thus far and confirms recent data from European Collaborative study of nine centres using the same marker in 350 families. We propose to continue investigation of linkage of AGT in this large sib-pair resource, however, current data does not support a major role for AGT in hypertension in white Europeans. This data does not exclude a small effect of AGT haplotypes that might only be detectable in case-control association studies.

Polymorphism of the MPO gene in patients with Stroke and Ischemic Heart Disease. *B. Nikpoor, G.A. Rouleau.* Neurogenetics Lab, Research Institute, Montreal General Hospital, 1650 Cedar Ave, Montreal, Quebec, Canada.

The role of the inflammatory pathway in the development of atherosclerosis has been poorly studied. Oxidation of the low density lipoprotein (LDL) by free radicals has a significant role in the inflammatory pathway and one important enzyme involved in this process is myeloperoxidase (MPO). This enzyme is involved in the production of free radicals in many cells. Active MPO is detected in the atherosclerotic lesion and believed to be the source of oxidants responsible for oxidation of LDL. There is a polymorphism in the promoter region of the gene which affects its transcription. There are two alleles, A and G, for this polymorphism. People with the G allele have a higher level of expression of the MPO than those who have the A allele. Considering the role of MPO in the process of atherosclerosis and the effect of the polymorphism on the activity of MPO, studying the relationship between this polymorphism and the incidence of the IHD and CVA is important. We used a case control design in order to study any possible associations, between the A and G polymorphisms and the incidence of IHD and CVA in the French Canadian population of Quebec. Preliminary results using only a portion of the total number of patients and controls expected to participate in our study are as follows: Among 159 control population, 9.4% inherited two copies of the A allele. In the group of 80 patients with MI 6% had the A/A genotype (odds ratio OR=.64, C.I.=.195-1.977) and in the CVA group 6% of 50 people had the A/A genotype (O.R =.613 C.I.=.134-2.396). Although the OR in the both groups has a range which includes 1 and therefore is not significant, but it is because of the small number of cases which do not have enough statistical power for detecting significant difference between cases and controls especially for this allele which has a low frequency. Because of the promising results of the pilot stage now we are recruiting 2 groups of French Canadian patients with IHD and CVA from 2 different locations and the results of the next stages will be released soon.

A genome-wide linkage analysis of left ventricular diastolic early filling velocity in hypertensive African-Americans and Whites: The NHLBI Family Blood Pressure Program HyperGEN Echocardiography Study. *W.*

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Left ventricular (LV) diastolic dysfunction is an important precursor for heart failure. Evidence suggests that LV diastolic function is under genetic control, independent of risk factors known to influence LV size and geometry. Left ventricular transmitral peak early filling velocity (MVE) is a functional index of left ventricular active relaxation at early diastole. We conducted genome-wide scan for MVE as part of the HyperGEN Echocardiography Study. HyperGEN is one of four networks in the NHLBI Family Blood Pressure Program. Doppler echocardiography was performed in four field centers in hypertensive sibships. This analysis was conducted on 167 Caucasian (398 subjects, mean age=60 years) and 183 African-American (395 subjects, mean age=52 years) hypertensive sibships. MVE was adjusted for age, age² and heart rate in race and sex specific linear regression models and standardized residuals were calculated. Multipoint linkage analysis was conducted using a variance-components approach (GENEHUNTER) with 374 anonymous markers of an average marker density of 10 cM (CHLC8). A maximum LOD score of 4.26 was obtained on chromosome 5 at 133.6 cM between markers D5S1505 (LOD=3.16) and D5S816 (LOD=2.98) in African-Americans. Suggestive evidence for linkage was observed for regions on chromosome 20 (LOD=2.03 at D20S103) in African-Americans and chromosome 18 (LOD=1.92 at D18S535) in Caucasians. In conclusion, our study suggests several regions that may contain genes controlling LV diastolic early relaxation.

Full Genome Scan of HDL-cholesterol in the NHLBI Family Heart Study (FHS). *J.M. Peacock¹, D.K. Arnett¹, L.D. Atwood¹, R.H. Myers², M.A. Province³, H. Coon⁴, S.S. Rich⁵, G. Heiss⁵*. 1) Division of Epidemiology, School of Public Health, University of Minnesota, Minneapolis, MN; 2) Section of Preventive Medicine and Epidemiology, Boston University, Boston, MA; 3) Division of Biostatistics, Washington University Medical School, Saint Louis, MO; 4) Department of Psychiatry, University of Utah, Salt Lake City, UT; 5) Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC.

The quantitative trait HDL cholesterol (HDL-c) is a strong independent predictor of coronary heart disease (CHD). HDL-c is highly heritable and known environmental predictors explain only a small portion of its total phenotypic variance. Many studies have assessed linkage of candidate gene loci with HDL-c, but relatively few have attempted to identify new chromosomal regions that influence this trait. We analyzed 872 caucasian individuals from 105 families taking part in the NHLBI Family Heart Study (FHS), which was conceived to examine important environmental and genetic predictors of CHD and associated risk factors. One primary goal of FHS is to locate genes that influence major CHD traits by performing a genome wide anonymous marker scan. In order to maximize the relative contribution of genetic components of variance to the total variance of HDL-c, the HDL-c phenotype was regressed on age, age², waist-hip ratio (WHR), subscapular skinfolds, and FHS field center. Standardized HDL-c residuals were calculated separately for men and women. All analyses were completed using the variance components method, as implemented in the program GENEHUNTER using 369 anonymous markers from the CHLC10 screening set. The heritability of residual HDL-c in this sample was 53%. Significant linkage of residual HDL-c was detected near marker D5S1470 at location 41.7 cM on chromosome 5 (LOD = 3.4). Suggestive linkage was detected near marker D13S1493 at location 29.3 cM on chromosome 13 (LOD = 2.5). We conclude that at least one genomic region is likely to harbor a gene that influences interindividual variation in HDL cholesterol.

Familial juvenile hyperuricemic nephropathy and autosomal dominant medullary cystic kidney disease type 2: two facets of the same disease? K.S. Dahan¹, A. Fuchshuber², S. Adamis¹, M. Smaers¹, S. Kroiss², G. Loute³, J.P.

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Familial juvenile hyperuricemic nephropathy (FJHN) is an autosomal-dominant disorder heralded by hyperuricemia during childhood, characterized by a chronic interstitial nephritis with marked thickening of tubular basement membranes, leading to progressive renal failure during adulthood. A gene for FJHN was recently mapped on chromosome 16p11.2 in Czech families (Stiburka B. et al: *Am J Hum Genet* 66;1989-1994, 2000), close to the *MCKD2* locus, which is responsible for a variant of autosomal dominant medullary cystic disease (ADMCKD) (Scolari F. et al: *Am J Hum Genet* 64; 1655-1660, 1999). It raises the question as to whether two distinct genes co-localize within the 10 cM region delimited by the authors or whether these two disorders are actually the same. In a large Belgian family with FJHN we confirm a tight linkage between the disorder and the marker D16S3060 on chromosome 16p12 (maximum two-point LOD score 3.74 at recombination fraction (qmax) of 0. We further narrow the candidate region to a ~2 cM interval between D16S3060 and D16S3036. Moreover, we found a striking clinical and pathological resemblance between ADMCKD and the FJHN occurring in our family, including the presence of medullary cysts. Our findings strongly suggest that FJHN and ADMCKD2 are allelic variants of the same disease entity.

Haplotype association of chromosome 11q Bardet-Biedl syndrome locus and elevated leptin values and obesity in BBS families and non-BBS control group in Puerto Rican population. *S. Carlo*¹, *J. Acevedo*², *D. Valencia*³, *I. Ramos*³, *V. Franceschini*³, *C. Rios*⁴, *T. Fraser*⁴, *V. Sheffield*⁵, *A.S. Cornier*³.

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Bardet-Biedl syndrome (BBS, MIM#209900) is an autosomal recessive disorder of unknown etiology that exhibits phenotypic and genetic heterogeneity. Characteristic features includes retinitis pigmentosa, obesity, hypogenitalism, post-axial polydactyly and mental retardation. We have studied 12 families with 34 affected individuals, 321 relatives (68 obligate carriers) and a control group of 100 individuals. All BBS families showed linkage to chromosome 11q locus. Haplotype characterization and linkage analysis was performed as well as determination of heterozygote status of the haplotypes associated with the disease. Characterization of the 11q region from GATA8A08 to PYGM markers resulted in multipoint linkage analyses of each family that generated independent LOD scores between 2.89 and 5.7. Heterozygotes for the BBS gene exhibited a 67% prevalence of obesity, 58% of diabetes, and 43% of hypertension. We determined leptin values and correlate it with their respective body mass indexes (BMI's). A strong association between a specific haplotype and elevated leptin values and obesity was achieved in 90.7% of the individuals. No significant differences were found in BMI median among patient, family members and control group, so forth their leptin values did not showed direct correlation with their BMI's as expected in general population. Instead significant elevation of leptin levels (at least three fold) was associated with a specific haplotype within the BBS region in patients, family members and control group individuals that exhibited the haplotype. We propose that having this specific haplotype for the BBS gene on chromosome 11q does predispose to higher leptin values and BMI's.

Refined linkage analysis of X-linked infantile spinal muscular atrophy (XL-SMA) families reveals recombination events which define the critical region. *L.L. Baumbach¹, D. Dressman^{2,3}, H. Basterrechea¹, R.D. Clark⁴, B. Wirth⁵, E. Hoffman².* 1) Univ Miami Sch Medicine, Miami, FL; 2) Children's National Medical Center, Washington, D.C; 3) Univ, of Pittsburgh, Pittsburgh, PA; 4) USC/Norris Comprehensive Cancer Center, Los Angeles, CA; 5) Univ. Bonn, Bonn, Germany.

We have previously reported an X-linked recessive form of infantile lethal motor neuron disease (MIM 30021), which closely resembles Werdnig-Hoffman disease, except for additional features of early onset or congenital contractures and/or fractures, in a single family that mapped to Xp11.3-Xq11.2. Here we report clinical and genetic data for eleven additional unrelated families identified in North America and Central and Western Europe. Of these, six families have been tested for linkage on the X-chromosome; all of these mapped to the same region as the first family described. One of these six families is thought to represent a new mutational event, and although was informative for the markers used, and highly suggestive of positive linkage to the same region, was not included in LOD score calculations. The cumulative LOD score for the five families is 7.3 at a $\Theta = 0.0$ in the Xp11.3-q11.2 region. Further multipoint linkage analysis using these families has narrowed the candidate disease gene interval to a region defined by DXS8035-DXS1194. **These results strongly support the existence of a major disease locus for XL-SMA between DXS 8026-DXS 7132 (Xp11.3-Xq11.2).**

There is a growing number of SMA patients testing "negative" for chromosome 5q *SMN* gene abnormalities. Our data suggests that XL-SMA may not be as rare as previously assumed; that families representing new mutations in the yet-to-be identified *XL-SMA* gene exist; and that patients testing negative for *SMN* mutations might instead be affected by XL-SMA. *This study has been generously supported by grants from the MDA, Patterson Trust, and Pentland- Hall Foundation, Dade County..*

A Novel Autosomal Dominant Nephropathy Collocalizes to the ADMCKD Locus on chromosome 1. *R. Parvari*¹, *A. Shnaider*², *A. Kanis*⁴, *Z. Borochovit*³, *D. Landau*². 1) Microbiol/Immunol/Fac Hlth Sci, Ben Gurion Univ of Negev, Beer-Sheva, Israel; 2) Nephrology, Soroka University Medical Center; 3) Fac health Sciences, Technion, Haifa, Israel; 4) Dept of Pediatrics, University of Iowa, IO, USA.

Familial tubulointerstitial nephropathies with adult onset, progressive deterioration and an autosomal dominant inheritance include mainly cases related to the medullary cystic disease (ADMCKD) complex, but families with a less defined familial interstitial nephritis have also been described. We studied a kindred of Jewish ancestry in whom 15 members (both males and females) have suffered from chronic renal failure over 4 consecutive generations, thus hinting for an autosomal dominant mode of inheritance. The first evidence for renal involvement could be seen as early as age 18 years and as late as age 38 years. It included hypertension, followed by progressive renal insufficiency, but no polyuria, anemia, hematuria or proteinuria. End stage renal disease developed at an average age of 33 years (range 20 to 48 years). Renal pathology at early stages of the disease showed extensive tubulointerstitial fibrosis and global glomerulosclerosis. Electron microscopy showed no evidence of glomerular immune complex deposition consistent with a primary glomerulonephritis. In addition, no glomerular basement membrane changes consistent with Alport syndrome were seen. Linkage analysis was performed to the two known loci of ADMCKD, on Chromosomes 1 and 16. Linkage between this disease and the chromosome 16 locus was excluded. However, linkage to the chromosome 1 locus of ADMCKD was established to 1q21 with a maximum two-point lod score of 3.80 to D1S394. The disease interval could be narrowed to about 5.5cM between D1S2858 and D1S2635. This is the first report of linkage of a familial nephropathy characterized by hypertension and progressive renal failure to the locus described for ADMCKD, a disease classically envisioned as a tubulointerstitial nephropathy, with salt loosing, anemia and normotension. This observation broadens the clinical spectrum of ADMCKD. The two diseases may be allelic or caused by different genes in the same, but yet large, chromosomal interval.

Genome Screen for a New HPS Gene in the Puerto Rican Pediatric Population. A.E. Maldonado¹, P.J. Santiago-Borrero², A. Gonzalez³, R.A. Spritz⁴, C.L. Cadilla¹. 1) Biochemistry; 2) Pediatrics; 3) Pathology Depts., UPR School of Medicine, San Juan, PR; 4) Human Medical Genetics Program, University of Colorado, CO.

Hermansky-Pudlak Syndrome (HPS) is an autosomal recessive disorder consisting of OCA, ceroid-like products deposition in tissues and a bleeding tendency due to storage pool-deficient platelets. HPS is frequently found in the Puerto Rican (PR) population and a village in the Swiss Alps. Using these populations the first gene associated with HPS (HPS1) was found by linkage disequilibrium mapping and positional cloning. The *pale ear (ep)* mouse mutant was identified to be the counterpart for human HPS1. A 16-bp duplication frameshift mutation in the HPS1 gene's exon 15 was found in the PR patients, which serves as a molecular diagnostic tool as well as analysis of platelets' d granules by Electron Microscope (EM). A second HPS gene (HPS2) has been identified and codes for the b3A-adaptin subunit of the Adaptor Protein Complex, which corresponds to the defective gene in the *pearl (pe)* mouse mutant. We have screened a total of 80 PR patients for the 16-bp duplication and identified 36 positive homozygotes, 9 heterozygotes and 35 individuals who lacked the duplication. EM analysis of platelets of some duplication-negative patients revealed a wide spectrum of dense bodies (0-8). Based on the molecular and clinical data, we selected 11 duplication-negative patients to perform exon screening by SSCP/HDX analysis and DNA sequence of HPS1 gene and linkage disequilibrium mapping. The SSCP/HDX and DNA sequence analysis showed no pathologic mutations, only naturally occurring polymorphisms. The possibility of having another non exonic mutation in the same locus was excluded by genotyping a polymorphic marker close to HPS1 (D10S184). The *ruby eye (ru)*, *reduced pigment (rp)*, *pallid (pa)*, *cocoa (coa)*, *light ear (le)*, *sandy (sdy)*, *muted (mh)*, *ruby eye-2 (ru-2)*, *gunmetal (gm)* and *subtle gray (sut)* homologous regions in the human genome equivalent to mouse HPS models did not showed excess homozygosity. Polymorphic markers in the vicinity of AP-3 d and m subunits were screened and excluded as candidates. Supported by NIH-RCMI-G-12-RR-0305, NIH-RO1-AR-39892 and UPR School of Medicine.

Evidence of genetic heterogeneity in fourteen cases of congenital generalised lipodystrophy. *M.A. Patton¹, A. Rajab², S. Joshi³, K. Heathcote¹, S. Jeffery¹.* 1) Medical Genetics, St George's Hospital Medical School, London, UK; 2) Genetic Unit, Ministry of Health, Sultanate of Oman; 3) Paediatrics, Sultan Qaboos University Hospital, Sultanate of Oman.

Congenital generalised lipodystrophy (Berardinelli-Seip syndrome OMIM no 269700) has recently been mapped by Garg et al to chromosome 9q34. Another form of partial lipodystrophy (Dunnigan syndrome OMIM no 151660) has been found to be due to mutations in exon 8 and exon 11 of the lamin A/C gene on chromosome 1q21. The lamin gene is also involved in the production of autosomal dominant inherited cases of Emery Dreifuss muscular dystrophy and Dilated Cardiomyopathy with conduction system disease. Fourteen cases of congenital lipodystrophy have been ascertained in the Sultanate of Oman. All cases are born to consanguineous parents. A search for homozygosity at 9q34 only revealed two cases compatible with the Berardinelli-Seip locus. Two pairs of sibs from the same tribal origin had features of muscle disease with cardiac enlargement, raised CK and EMG evidence of myotonia in addition to lipodystrophy. A search for homozygosity at the lamin locus and mutation screening in exon 8 of the lamin gene was negative. The results indicate there is further genetic heterogeneity in congenital generalised lipodystrophy and a genome wide screen for homozygosity is underway.

Fine mapping and searching for the gene for familial partial epilepsy with variable foci (FPEVF) on chromosome

22q12. *L. Xiong*¹, *P. Cossette*¹, *M. Labuda*¹, *T. Hudson*², *J. Mulley*³, *J. Serratosa*⁵, *D.S. Li*¹, *S. SeniMercho*⁴, *M.H. Seni*⁴, *S.F. Berkovic*⁴, *F. Dubeau*⁴, *F. Andermann*⁴, *E. Andermann*⁴, *M. Pandolfo*¹. 1) Notre-Dame Hospital, CHUM, Montreal, PQ., Canada; 2) Montreal General Hospital, McGill University, Montreal, PQ, Canada; 3) Womens and Childrens Hospital, North Adelaide, Australia; 4) Montreal Neurological Institute, McGill University, PQ, Canada; 5) Neurology Service, Foundation Jimenez, Madrid, Spain.

We recently mapped a new partial epilepsy syndrome (FPEVF) to chromosome 22q12 in two large French Canadian (FC) families. The syndrome is characterized by mainly partial nocturnal seizures arising from variable brain areas of the affected individuals without any evidence of structural brain damage or neurological impairment. Haplotype analysis and recombination events had flanked the candidate region to a ~5.6 Mb interval on 22q12. In order to identify more families with FPEVF, to further narrow down the candidate region, and eventually clone the causative gene, we developed a detailed physical map with saturated single nucleotide polymorphisms and microsatellite markers between markers D22S1144 and D22S280. We tested more FC and non-FC families with multiple cases of partial epilepsy for linkage to 22q12, determined the marker haplotypes and evaluated the phenotypes. We found out that six more FC families, one Spanish family with similar phenotype show evidence of linkage to chromosome 22q12. Homozygous individuals carrying two copies of the disease haplotype present with more frequent and intractable seizures. They all share a marker haplotype in a ~3.0 Mb region on chromosome 22q12 between markers D22S1150 and D22S1176, except for the Spanish family which has a different haplotype. we conclude that a gene determining a familial form of partial epilepsy is localized on chromosome 22q12. This gene is a frequent cause of idiopathic partial epilepsy not only in French Canadians, as shown by the identification of a linked Spanish family (lod score ~3). Haplotype data suggest that all FC families identified so far probably segregate the same mutation. A search for the responsible gene is in progress.

Linkage and comparative mapping of canine cone degeneration: Locus homology to Pingelapese achromatopsia.

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Canine cone degeneration (cd) and its human counterpart, achromatopsia, are characterized by day-blindness, absent retinal cone function and normal rod function by electroretinogram. Although cd is inherited as a simple autosomal recessive factor, human achromatopsia is a heterogeneous autosomal recessive disease caused by at least three loci. It is one of over 350 canine genetic diseases with human equivalents, many of which have no comparable phenotype in other species. The selective breeding practices employed in the development of purebred lineages, including frequent population bottlenecks and founder effects, have enriched purebred populations for recessive genetic diseases, allowing recognition of discrete genetic disorders and reducing the likelihood of locus heterogeneity for complex phenotypes.

We report linkage of the canine cd locus to marker C29.2 on canine chromosome 29 at theta = 0.0 with a maximum lod score of 23.78. Outbred pedigrees generated for use in this study were derived from cd-affected Alaskan Malamutes segregating the cd phenotype. Conserved synteny of the canine genome with the long arm of human chromosome 8 strongly suggests that the cd locus is homologous to the achromatopsia locus ACHM3 on human chromosome 8q21-22. Recent advances in the development of the canine genome map and its correlation to the human genome facilitate mapping of complex human genetic diseases. Cd is the third canine retinal degenerative disease mapped by our group. The mapping of cd to CFA 29 and determination of its homology to ACHM3 underscore the emerging power of canine genomics to aid in the pursuit of the genes causing human genetic disease.

Recovering inheritance information for linkage analysis in large pedigrees by Markov chain Monte Carlo multipoint computations. *A. Bureau¹, T.P. Speed^{1,2,3}, P.N. Baird⁴*. 1) Biostatistics, School of Public Health, University of California, Berkeley, CA; 2) Dept. of Statistics, University of California, Berkeley, CA; 3) Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia; 4) Center for Eye Research, Royal Victorian Eye and Ear Hospital, Melbourne, Victoria, Australia.

In large pedigrees, missing ancestral genotypes add to genotyping failures and imperfect marker informativeness to limit the available inheritance information for linkage analysis. Simultaneous analysis of multiple linked marker loci recovers much of the lost inheritance information in small pedigrees, and important information gains can be expected in large pedigrees as well. Since exact multipoint analysis is not computationally feasible on large pedigrees, we perform approximate computations using Markov chain Monte Carlo (MCMC) methods. Indicators of the grand-parental origin of alleles are used as latent variables. A combination of whole meiosis and whole locus Gibbs samplers and Metropolis steps involving multiple meioses achieves good sampling of the space of meiosis indicators for pedigrees up to at least 50 non-founders. A limited simulation study shows that the conditional expectation of allele sharing statistics given observed marker data becomes closer to the true allele sharing at a locus with a high sharing among affected individuals as the number of linked marker loci included in the analysis increases. The MCMC method is also applied to infer sets of individuals sharing an allele identical by descent. This is illustrated using actual genotype data around a locus where affected individuals in a large glaucoma pedigree are believed to have inherited an ancestral mutation.

Linkage mapping of Quantitative trait loci using allele sharing models in Allegro. *D.F. Gudbjartsson^{1,2}, G.M. Jonsdottir¹, G. Thorleifsson¹, A. Kong^{1,3}.* 1) Decode Genetics, Reykjavik, Iceland; 2) Institute of Statistics and Decision Sciences, Duke University, Durham, NC; 3) Department of Human Genetics, University of Chicago, Chicago, IL.

Variance components techniques and Haseman-Elston tests are popular methods of detecting linkage to quantitative trait loci (QTL). It has been shown that variance components methods are sensitive to misspecification of the phenotype sampling distribution, e.g. non-normality and selection, leading to an anti-conservative test (Allison et al 1999). The new Haseman-Elston test is more robust to model misspecification, but is not directly extendible to general families. Allele sharing models (ASM) have been used for mapping qualitative trait loci. We extend their application to QTL using a locally most powerful test statistic. The new method can be applied directly to general family structures and will be offered as a feature in our multipoint linkage program Allegro. As with qualitative traits, this test can always be calibrated to give proper type I error, irrespective of model misspecification. We present simulation results on both sib-pairs and more general family structures. Calibration and power of this test are compared with the other methods above. Simulations are performed assuming both complete and incomplete information on IBD sharing.

Performance of hybrid MCMC samplers on a large complex pedigree. *M. Szydlowski, S.C. Heath.* Department of Human Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY.

For large complex pedigrees and/or multilocus data Markov Chain Monte Carlo procedures may provide a foil to impractical exact methods of probability calculation. The sampling procedure may operate directly on the genotypic configuration or segregation indicators. The simplest samplers involve genotype-by-genotype or single segregation indicator sampling. These site-by-site samplers give highly autocorrelated samples making the analyses difficult in many cases. The whole locus sampler (L), which samples joint pedigree genotype configurations within a locus, significantly improves mixing properties. However, we show that the autocorrelation problem still remains for tightly linked loci. For this case, the whole meiosis sampler (M), which samples a joint segregation indicator pattern for all loci simultaneously, can have advantage over the L sampler. However, for a particular pedigree and available data pattern, the M sampler may be reducible, and is often much more time consuming than the L sampler. To provide a more general approach, hybrid samplers (LM) have been proposed which use both the L and M samplers. The LM samplers have potential to improve mixing properties and speed up multilocus analyses. To test this, we consider the properties and efficiency of LM samplers for analyses of codominant markers on a large complex pedigree of 2385 individuals from the Island of Kosrae. We demonstrate that the execution times and mixing properties of both L and M samplers are case dependent, and that the proportion of L and M steps in the hybrid sampler can be optimized to improve performance. Results on the application of LM samplers with different proportion of L and M steps are presented for various data sets and some recommendations on the use of the hybrid LM sampler is given. This work was supported by NIH grant GM58757-01.

Absolute Bitter-Blindness: A novel gustatory familial trait. *P.A.S. Breslin¹, C.D. Tharp¹, D.R. Reed¹, T. Huque¹, J.G. Brand¹, G.K. Beauchamp¹, A.I. Spielman².* 1) Monell Chemical Senses Ctr, Philadelphia, PA.19104; 2) New York Univ., College of Dentistry, New York, NY.10010.

The study of taste genetics in humans has long focused on individual differences in sensitivity to the bitter -N=C-S containing anti-thyroid drugs phenylthiocarbamide (PTC) and propylthiouracil (PROP). In the present study, we introduce a novel bitterness-sensitivity trait carried in a large family that spans four generations. A small proportion of family members possess a total inability to perceive bitterness from a broad range of structurally diverse "bitter-tasting" compounds (complete bitter blindness). There also appears to be members of this family who are partially bitter blind; selected compounds, particularly caffeine and sucrose octa-acetate (SOA), are not perceived as bitter. Finally, some family members perceive bitterness in an ordinary fashion. The trait does not follow simple Mendelian inheritance, nor is it sex-linked, as men and women within the family fall into all three bitter taster categories. Given the recent report of a large family of bitter receptor genes, it is unlikely that total bitter-blindness is caused by deleterious variation in each bitter receptor gene. Rather, the effect is likely determined downstream of the receptor at the level of the bitter-taste receptor cell (e.g., receptor cofactors, transduction components, enzymes, etc.) or higher in the CNS. In accord with this idea, one bitter-blind family member who donated fungiform gustatory tissue was found to possess mRNA at the predicted sizes for each of eight selected bitter receptors. A common splice component(s), enabling a single deleterious point mutation to affect all the receptors, is unlikely, since the published bitter-receptor genes are intronless. We are presently sequencing this individual's receptors and are conducting linkage analyses on this family and one other family, which carries a similar trait, to identify the gene(s) responsible for absolute bitter blindness. This work was supported by grants R29 DC02995 (PASB), R03DC50355 & R01DC00082 (DRR), and DC03969 (JGB).

Refined physical mapping of the Meckel syndrome gene (MKS2). *Y. Sirot¹, L. Benedetti-Cleeh¹, J. Augé¹, J. Roume², V. Cormier-Daire¹, M. Le Merrer¹, G. Gyapay³, A. Munnich¹, M. Vekemans¹, T. Attié-Bitach¹.* 1) dep de genetique-INSERM U-393, Hopital Necker-Enfants Malades, Paris, Paris; 2) service de foetopathologie hopital saint Antoine, Paris, France; 3) centre national du séquençage, Génoscope, Evry, Paris.

Meckel syndrome (MKS) is a rare, lethal, autosomal recessive congenital malformation characterized by occipital meningoencephalocele, multicystic dysplasia of the kidney, cystic and fibrotic changes of the liver, and polydactyly. So far, two MKS loci have been mapped to chromosomes 17q21-23 and 11q13 in finnish (MKS1) and african-middle eastern families respectively (MKS2). In an attempt to identify the MKS2 gene, we have constructed a BAC contig across the 0 cM critical region between markers D11S4079 and D11S937. The coverage of this region started by screening the RPCI-11 BAC library and is now based on using (i) the working draft database, (ii) fingerprinting analysis of the Sanger Institute database and (iii) BAC end-sequences of the RPCI-11 BAC library provided by the TIGR centre. In addition, to narrow down the MKS2 homozygous region, we studied novel CA repeats identified by the analysis of the sequenced BAC. In conclusion, we positioned six genes, six EST and several STS in the region by analysing the sequenced BACs of the contig. So far, two candidate genes were excluded by RT-PCR sequencing of two patients namely the p21-activated serine-threonine kinase gene PAK1 and the pICln gene involved in RNA processing. Other expressed sequences are being tested by the candidate gene approach.

A BAC/PAC contig of a region of chromosome 1q42-q43 containing a putative susceptibility gene for type 1 diabetes. *K. Gogolin Ewens¹, K. O'Brien¹, S. Gutin¹, A. Bruzel², S. Gregory³, P. Concannon⁴, R.S. Spielman¹.* 1) Dept Genetics, Univ Pennsylvania School of Medicine, Philadelphia, PA; 2) Dept Pediatrics, Univ Pennsylvania School of Medicine, Philadelphia, PA; 3) The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, UK; 4) Molecular Genetics, Virginia Mason Research Center, Seattle, WA and Univ Washington School of Medicine, Seattle, WA.

In spite of exhaustive studies of the genetic factors contributing to susceptibility to type 1 (insulin dependent) diabetes mellitus (T1DM or IDDM), the HLA region on 6p and IDDM2 near the insulin gene on 11p remain the only well-established T1DM susceptibility genes. A genome-wide screen of 428 multiplex families (467 ASPs) confirmed these two linkages and provided suggestive evidence for linkage of T1DM to a region of chromosome 1 (1q42-q43) centered on the polymorphic marker D1S1617 (lod=3.31) (Nature Genet, 19:292-296, 1998). In order to identify candidate genes in this region, we generated a high-density physical map of the 7-10 Mb region between D1S439 and D1S459, which flank D1S1617. Initially 104 BAC clones were isolated by screening the RPCI-11 BAC library with 40 STS markers mapping to this region. An additional 309 PAC clones were mapped to this region by The Sanger Centre. These clones were assembled into contiguous sets (contigs) by STS content analysis using a total of 111 STS markers. The order of clones within the contigs was verified by further STS content mapping using end-sequences derived from the BAC and PAC clones and by fingerprint mapping.

This BAC/PAC genomic contig is being used for sequencing and further characterization of the genomic structure of this region. Both molecular and electronic gene-finding techniques are being applied to identify the genes in this region and mutation screens will be carried out in diabetic families on any genes that appear to be likely candidates for T1DM.

Identifying the genetic locus for Duane syndrome with radial ray anomalies (Okihiro syndrome). *E.C. Engle^{1,2}, N. McIntosh², J. Fain², J. Martonyi³*. 1) Harvard Medical School, Boston, MA; 2) Children's Hospital, Boston, MA; 3) University of Michigan, Ann Arbor, MI.

Individuals affected by Duane syndrome with radial ray anomalies (Okihiro syndrome) are born with Duane syndrome, a non-progressive eye movement disorder exhibiting restrictions of lateral gaze with globe retraction on attempted adduction, and associated anomalies of the arm and/or hand (short or absent forearms, thenar eminence hypoplasia, hypoplastic or absent thumb or hypoplastic or absent radial or ulnar bones). Okihiro syndrome was first described in a family in 1977 by Okihiro et al. and is inherited in an autosomal dominant fashion. We are studying the clinical presentation and genetics of two families with Okihiro syndrome: a large family spanning three generations and consisting of 8 affected and 13 unaffected members, and a smaller branch of the original Okihiro family consisting of 2 affected and one unaffected member. Linkage analysis of the larger family shows that their disease gene does not map to: 1) the locus for isolated Duane syndrome on chromosome 2q31; 2) the region deleted in an individual with BOR/Duane syndrome on 8q12.2-21.2; 3) the region associated with a deletion in an individual with Duane syndrome on 4q27-31; 4) chromosome 22 (which as a derivative marker chromosome has been found in some individuals with Duane syndrome); or 5) any of the congenital fibrosis of the extraocular muscle loci (CFEOM1-3). We are performing a genome wide screen to identify the map location of the disease gene for Okihiro syndrome in these families. Assuming full penetrance of the Okihiro gene, the larger pedigree provides 13 informative meioses with a maximum theoretical lod score of 4.17.

A genome-wide search for linkage to atopic dermatitis and underlying quantitative traits. *W.O. Cookson¹, B. Ubhi¹, A. Walley¹, R. Lawrence¹, H. Cox^{1,2}, R. Coleman², G.R. Abecasis¹, M.F. Moffatt¹, J.I. Harper².* 1) WTCHG, University of Oxford, Oxford, England; 2) Institute for Child Health, Great Ormond Street Hospital, London, England.

Atopic Dermatitis (Eczema) affects one child in 7 in developed countries. It is part of the syndrome of atopy, which includes asthma and is typified by Immunoglobulin E responses to common respirable allergens. Segregation analyses indicate the presence of genes of strong effect underlying the syndrome, with the expectation that these genes may be identified by positional cloning. We have therefore carried out a genome-wide screen for genetic linkage to atopic dermatitis and its associated traits in a panel of 150 nuclear families identified through children attending tertiary referral clinics for the treatment of severe atopic dermatitis. The families contained 610 individuals. The children with eczema had significantly higher levels of the atopic RAST Index and the total serum IgE than children without disease (5.08 vs 1.51, $p < 0.0001$, and 450 vs 46.5, $p < 0.0001$ respectively). Sixty percent of children with eczema also has asthma. The families were initially screened with 347 markers, giving rise to 186666 genotypes. Preliminary sib-pair linkage analysis suggests linkage of asthma to chromosome 1q1-2 ($p = 0.0007$) and eczema to chromosome 20p ($p = 0.001$). Linkage to quantitative phenotypes will be sought by variance components analyses.

Fine genetic mapping of the 1q44 locus for familial cold urticaria. *H.M. Hoffman¹, F.A. Wright³, D.H. Broide¹, A.A. Wanderer⁴, R.D. Kolodner^{1,2}.* 1) Department of Medicine, University of California, San Diego, La Jolla, CA; 2) Ludwig Institute of Cancer Research and Cancer Center, University of California, San Diego, La Jolla, CA; 3) Division of Human Cancer Genetics and Cancer Center, Ohio State University, Columbus, OH; 4) Department of Pediatrics and Allergy, University of Colorado Health Sciences Center, Denver, CO.

Familial cold urticaria (FCU) is a rare hereditary inflammatory disease characterized by intermittent episodes of rash, fever, arthralgia, and conjunctivitis following generalized exposure to cold environments (OMIM 120100). The mode of inheritance is autosomal dominant with complete penetrance, although sporadic cases have occurred. We recently reported linkage for the FCU locus to a 10 cM interval between D1S423 and D1S2682 on chromosome 1q44 in five North American families. In order to further define this region, we genotyped these families and several additional subjects from FCU families using 5 additional short tandem repeat polymorphic markers in the interval. Marker order was established using radiation hybrid mapping. Two-point linkage analysis using MLINK revealed a maximum LOD score of 10.5 (recombination fraction = 0) at marker AFMA264ZC5. The proximal limit of the FCU locus was further defined through haplotype analysis which demonstrated a recombination event between AFMB358WG1 and AFMA264ZC5. This data establishes more firmly the region containing the gene for FCU, which will ultimately lead to the identification of the genetic and molecular basis of this unusual inflammatory disease.

Mapping a dominant form of multinodular goiter to chromosome Xp22. *F. Capon¹, A. Tacconelli¹, S.*

Sciacchitano², R. Bruno³, V. Tassi⁴, V. Trischitta⁴, S. Filetti⁵, B. Dallapiccola², G. Novelli¹. 1) Dept Biopathology, Tor Vergata Univ Rome, Roma, Italy; 2) Dept. of Experimental Medicine and Pathology, La Sapienza Univ Rome, Roma, Italy; 3) Hospital of Tinchì (MT), Italy; 4) Endocrinology Unit, CSS IRCCS, San Giovanni Rotondo (FG), Italy; 5) Dept of Experimental Medicine, Univ Catanzaro, Italy.

Diffuse or nodular enlargement of the thyroid gland that is not the result of an inflammatory or neoplastic process (thyroid goiter) is a common disorder characterized by a 5 to 1 female to male ratio. Even if the disease is associated with iodine deficiency, familial forms of goiter have also been reported in areas where iodine intake is ample. A genome scan of one such pedigree has mapped a locus for multinodular goiter (MNG1) to chromosome 14q. Moreover gene mutations impairing the thyroid hormonogenesis have been identified in patients affected by different forms of goiter. We report here the analysis of an Italian three-generation MNG pedigree, including 10 affected females and 2 males. First, we excluded linkage to the MNG1 locus and to 8 additional candidate regions harboring 9 genes involved in thyroid hormone biosynthesis. Then, we undertook the search of a novel MNG locus. Since, no male-to-male transmission was present in our pedigree, we hypothesized an X-linked dominant pattern of inheritance. Thus, we examined 18 markers spaced at 10 cM intervals on the X chromosome. A significant lod score was observed in the Xp22 region, where DXS1226 marker generated a Z_{max} value of 4.73 at q = 0. Analysis of 4 flanking microsatellites confirmed this data, and haplotype inspection delimited a 12 cM candidate region lying between DXS7105 and DXS1214. Work funded by the Italian Telethon (grant E1031).

Genome-wide Scan for Dehydroepiandrosterones Measured Before and After an Exercise Training Program in the HERITAGE Family Study. *P. An¹, T. Rice¹, R. Rosmond², O. Ukkola², J. Gagnon¹, T. Rankinen², A.S. Leon³, J.S. Skinner⁴, J.H. Wilmore⁵, C. Bouchard², D.C. Rao¹.* 1) Division of Biostatistics, Washington University School of Medicine, Saint Louis, MO; 2) Pennington Biomedical Research Center, Baton Rouge, LA; 3) School of Kinesiology and Leisure Studies, University of Minnesota, Minneapolis, MN; 4) Department of Kinesiology, Indiana University, Bloomington, IN; 5) Department of Health and Kinesiology, Texas A&M University, College Station, TX.

A genome-wide search was performed for finding genes influencing dehydroepiandrosterone (DHEA), its fatty acid ester (DHEAE), and DHEA sulfate (DHEAS) at baseline and in response to endurance exercise training in 99 sedentary Caucasian families who participated in the HERITAGE Family Study. A total of 324 polymorphic markers covering all 22 pairs of autosomes were employed. Levels of DHEA, DHEAE, and DHEAS were not normally distributed and were thus log transformed prior to adjusting for age, sex, BMI, and levels of testosterone, estradiol, SHBG, and fasting insulin (training responses, defined as post-training minus baseline, were additionally adjusted for baseline values). Multipoint variance component linkage analyses using the computer program SEGPATH were performed. We found four significant linkages for baseline DHEAE, with LOD scores over 3.63 ($P < 0.000022$): at D1S551 (97.853 cM; 1p22.1), at D1S468 (2.533 cM; 1p36.32), at D1S1612 (4.675 cM; 1p36.32), and at the EPO locus (108.392 cM; 7q22.1). We also found six other linkages with LODs over 3.00 ($P < 0.0001$) for baseline DHEAE on 3p23 (D3S2432), 4p15.2 (D4S2397), 7q21.3-q22.2 (D7S1799, D7S821, PON1), and 12p13.33 (D12S372). Results with suggestive evidence of linkage (LODs over 1.75 and $P < 0.0023$) for baseline DHEAS were detected in four cases near chromosomes 3p21.1-p21.31, 8q21.3, and 21q13.1. Moreover, linkage results from the present study for baseline DHEAS and its training response (or its response to training) with markers on 19q12-q13.3 (INSR, D19S215, D19S433, $P = 0.0072 \sim 0.0109$) support previous genetic linkage mapping of STD. Further studies of candidates in the HERITAGE families are warranted in these chromosomal regions.

No linkage of a *FASL* microsatellite marker with type 1 (insulin dependent) diabetes in Danish families. R.

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Type 1 (insulin dependent) diabetes mellitus is the result of an immune mediated selective destruction of the pancreatic beta cell. There is evidence that Fas ligand (FasL) mediated apoptosis may play a role in the pathogenesis of type 1 diabetes, probably on the level of the T cell. The Fas/FasL mediated apoptosis is important in maintaining peripheral self-tolerance and in down regulating an immune response. The human *FASL* was therefore evaluated as a candidate susceptibility gene for type 1 diabetes using a microsatellite located in the 3' UTR of *FASL* on chromosome 1q23.1. A Danish type 1 diabetes family collection of 1143 individuals comprising 257 families (420 affected and 252 unaffected offspring) were typed for the polymorphic marker with 7 alleles observed, ranging from 14 to 22 GT repeats. Data were analyzed for linkage using the extended transmission disequilibrium test, ETDT. Parental heterozygosity index was found to be 0.32. The transmission frequencies of the most frequent allele, 16 repeat units, from heterozygous parents were 58% (117 transmitted and 85 not transmitted) and 54% (61 transmitted and 51 not transmitted) to affected and unaffected offspring, respectively. An inverse transmission pattern was found for the second most frequent allele with 17 repeat units; 43% (78 transmitted and 102 not transmitted) and 42% (42 transmitted and 58 not transmitted) of this allele were transmitted to affected and unaffected offspring, respectively. In conclusion, we find no evidence for linkage of the *FASL* microsatellite to type 1 diabetes in a Danish type 1 diabetes family collection. We found however preferential meiotic segregation of the 16 repeat allele both to affected as well unaffected offspring (58% versus 54%) and this shows the importance of testing transmissions to unaffected as well as affected offspring.

Improved Relationship Inference for Pairs of Individuals. *M.P. Epstein, W.L. Duren, M. Boehnke.* Department of Biostatistics, University of Michigan, Ann Arbor MI.

Valid inference of genetic linkage requires correct relationship specification between the different pairs of individuals in the analysis. Misclassification of relationships can lead to reduced or inappropriately increased evidence for linkage. Boehnke and Cox (1997) introduced a likelihood-based method for inferring the most likely relationship for putative sib pairs. They calculated the multipoint probability of the marker data for each pair conditional on each of four possible relationships: full sibs, MZ twins, half sibs, and unrelated pairs. To do so, they assume no genetic interference so that identity-by-descent states at an ordered map of markers represent a nonhomogeneous Markov chain. The multipoint probability depends on population marker allele frequencies, intermarker distance, and the presumed relationship of the pair.

We have improved and extended this method in several ways. First, rather than considering only putative sib pairs, we test all possible pairs of individuals in our sample. This may identify related individuals erroneously classified as unrelated due to sample switches or duplications. Second, we test other relationships; parent-offspring, grandparent-grandchild, avuncular relationships, and first cousins. Third, we allow for X-linked marker data in the multipoint probability calculations. For specific relationship-gender combinations, X-linked data can be particularly helpful.

Using simulated autosomal genome scan data, our method has excellent power to differentiate MZ twins, full sibs, parent-offspring pairs, second-degree relatives, first cousins, and unrelated pairs, but is unable to distinguish accurately among the second-degree relationships of half sibs, avuncular pairs, and grandparent-grandchild pairs. X-linked data improves our ability to discriminate among certain gender-specific types of second-degree relationships. We have included these extensions in our latest version of our computer program RELPAIR, which can be downloaded free of charge at www.sph.umich.edu/statgen/software.

A search for genetic loci involved in predisposition to bipolar mood disorder in the population of Antioquia, Colombia. *L.G. Carvajal-Carmona¹, J. Ospina-Duque², J. Calle², C. Lopez², L. Ochoa², J. Garcia², D. Ortiz-Barrientos², N. Pineda², A. Ospina², M. Lopez², A. Gallo², A. Miranda², M. Cuartas², P. Montoya², C. Palacio², G. Bedoya², M. McCarthy³, V. Reus⁴, N. Freimer⁴, A. Ruiz Linares^{1,2}.* 1) Department of Medical and Community Genetics, Imperial College, U.K; 2) Facultad de Medicina, Universidad de Antioquia, A.A. 1226 Medellin, Colombia; 3) Imperial College Genetics and Genomics Research institute, Imperial College School of Medicine (Hammersmith Campus), London, U.K; 4) Department of Psychiatry, UCSF, San Francisco, U.S.A.

We are collecting a bipolar disorder type 1 study sample from the province of Antioquia in Colombia. Historical demography data and genetic founder effects indicate that this population constitutes an internal isolate. Through review of clinical records and family interviews, 22 nuclear families with multiple cases of bipolar 1 have been identified. Extension of pedigrees has detected 227 living individuals with psychiatric disorders. Patients are being assessed using the Diagnostic Interview for Genetic Studies (NIMH) and a best estimate procedure. Thus far 76 individuals have been confirmed as bipolar 1, 3 as bipolar 2, 22 as major depression and 23 have other psychiatric diagnoses. A further 98 sporadic bipolar 1 cases have been identified and confirmed by DIGS. An estimate of the power to detect linkage to a major locus in the current pedigree set was obtained by simulation. The model used assumes a disease allele frequency of 0.003, penetrances set to 0.01, 0.81 and 0.9 (for genotypes NN, ND and ND) and a 4 allele marker at 5cM from the disease locus. These simulations indicate that, in the best-documented pedigrees, the current power to detect a lod score greater than 1 ranges between 33 and 86%. Two of these pedigrees share common ancestors six generation back and include 25 individuals with bipolar I and 35 individuals with other psychiatric conditions. This extended pedigree has a combined power to detect a lod score greater than 3 of 55%. This work was supported by Universidad de Antioquia (CODI grant #9845), Colciencias (grant #1115-04-414-98) and the Wellcome Trust (grant #056081).

Fine Mapping and Genetic Heterogeneity in Autosomal Dominant Familial Spastic Paraplegia. *P.C. Gaskell¹, A. Ashley-Koch¹, E.R. Bonner¹, S.G. West¹, C.M. Wolpert¹, C. Warner², C.D. Farrell³, I.K. Svenson¹, D.A. Marchuk¹, R.W. Tim¹, R.M. Boustany¹, J.M. Vance¹, W.K. Scott¹, M.A. Pericak-Vance¹.* 1) Duke University Medical Center, Durham, NC; 2) Dent. Neurologic Group, Buffalo, NY; 3) Roswell Park Cancer Institute, Buffalo, NY.

We evaluated seven families segregating pure, autosomal dominant familial spastic paraplegia (SPG), previously unlinked to SPG loci on chromosomes 2q, 14q, and 15q, for linkage to four recently identified SPG loci on chromosomes 2p (Fontaine et al, 2000), 8q (Hedera et al, 1999), 12q (Reid et al, 1999), and 19q (Reid et al, 2000). Two point heterogeneity analysis was significant for chromosome 12q (D12S368: $p=0.02$, D12S1691: $p=0.005$). Lod scores for family 3 were positive for chromosome 12q, peaking at D12S1691 (multipoint lod=3.22). Haplotype analysis of family 3 did not identify any recombinants in the 12q candidate region among affected individuals. Two point heterogeneity analysis for chromosome 19q was suggestive of linkage heterogeneity at marker D19S868 ($p=0.08$). Family 5 yielded a peak two point lod score of 2.02 at marker D19S868. The maximum expected lod for this family, determined by SIMLINK, was 1.9. Haplotype analysis of family 5 revealed crossovers in affected individuals at markers D19S220 and D19S420, thereby narrowing the most likely placement of the putative gene (SPG12) to a 5 cM region between markers D19S868 and D19S220. There was no evidence for linkage of any of the families to the chromosome 2p or 8q loci. Two of the families excluded all four loci examined, providing evidence for further genetic heterogeneity of autosomal dominant SPG. Of the remaining three families, two were uninformative for the chromosome 2p locus and one was uninformative for the chromosome 2p and 12q loci. The five families presently unlinked to all known SPG loci are presently being screened to identify novel SPG loci. In conclusion, these data confirm the presence of a chromosome 12 SPG locus, reduce the minimum candidate region for a chromosome 19q SPG locus, and suggest that at least one additional autosomal dominant SPG locus exists.

A new locus for autosomal recessive cone-rod dystrophy maps to Chromosome 8p12-q11 distal to RP1.. M.

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Inherited retinal degenerations have an incidence of approximately 1 in 4,000, and include forms with different pathologies many of which lead to blindness. Among the most common are: retinitis pigmentosa (RP), cone-rod dystrophy (CRD), and macular dystrophy. To date, a total of 122 loci carrying gene mutations causing various forms of retinal degeneration have been mapped, and the mutant gene has been identified in 56 of them (RetNet). Among these are only 3 loci for autosomal recessive (ar) CRD. Here we report a new fourth locus in a consanguineous Brazilian family with severe arCRD.

Genomic DNAs from 23 members of the family were genotyped by the Marshfield Mammalian Genotyping Service for over 400 high-quality short tandem repeat polymorphisms covering the entire genome with an average spacing of approximately 9 cM. The data were input directly to the pedigree information package Cyrillic which prepared output files for the LINKAGE program suite used to calculate lod scores. Significant linkage in the region 8p12-q11 was identified prompting the genotyping of a number of additional markers. Four-point linkage analysis with D8S1048, D8S1477 and D8S1810 give a maximum lod score of 6.13 at D8S1810. Haplotype analysis defined an interval of approximately 23cM between markers D8S560 and D8S589 which is immediately adjacent to but distinct from the RP1 locus for dominant RP. Only one marker so far studied in this interval (D8S1810) is homozygous in affected individuals. This implies that the defective gene in this consanguineous family is close to this marker. However, it is also possible that the patients are compound heterozygotes for different mutations in the responsible gene.

In summary, we have identified a new genetic form of autosomal recessive cone-rod dystrophy which is due to a yet unidentified gene in the interval D8S560-D8S589.

Affected-Sibship Statistics for Nonparametric Linkage Analysis. *H. Sengul, D.E. Weeks, E. Feingold.* Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

The literature on "nonparametric" linkage analysis includes a plethora of different statistics for scoring IBD sharing in affected sibships. We have reviewed the literature and listed almost twenty distinct statistics that apply to sibships of any size. For each statistic, we tabulated the values it assigns to each IBD sharing configuration in sibships of sizes two, three, four, and five. In addition, we calculated the mean and variance of each statistic in each sibship size. Finally, we compared the power of all of the statistics under thirty different genetic models. For families of two and three affected children, most of the statistics are identical, although a few take on different values that give them more or less power than the others under various models. For families of four and five affected siblings, however, there is more variety in the values of the statistics and thus more variation in power.

A Genome-scan for linkage of Specific Language Impairment: Report on chromosomes 1, 6, and 15. C.W.

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Specific Language Impairment (SLI) is a complex cognitive disorder characterized by a failure to develop language normally in the absence of hearing, neurological, or psychiatric difficulties. Since as many as 80% of persons with SLI are later diagnosed with dyslexia, the genome-scan for linkage to SLI was begun on chromosomes with previous suggestive reports of loci for dyslexia susceptibility. Fourteen families (n = 93 subjects) were initially genotyped using markers at approximately 10 cM intervals. LOD scores over 1.5 were then followed up by genotyping a denser marker map in the original families and in 5 additional larger families (n = 93 subjects). Multipoint linkage analysis of markers on chromosomes 1, 6, and 15, using categorical affection status of language impairment from clinical assessment, reading discrepancy, and the Language Standard Score failed to produce any LOD score over 1.5 (all families combined). Simulation studies empirically confirmed that this failure to detect linkage was not caused by a lack of statistical power in these families. Quantitative trait linkage analysis was then conducted using several reading and language measures analogous to tests used in previous dyslexia studies. Analysis on chromosome 15 using a word identification task produced a maximum LOD score over 2 in a set of twelve families. This region overlaps an area previously reported as linked to a similar measure in families with dyslexia. These findings suggest that ascertaining SLI families via a proband child may select a population that has some genetic overlap with dyslexia. An approximate 10 cM resolution genome scan is currently in progress with 82% of the markers finished on the initial set of families, with no two-point LOD score > 1.8 identified to date.

Linkage analysis of schizophrenia in isolated Daghestanian kindreds. *K. Bulayeva*¹, *B. Devlin*², *S-A. Bacanu*², *P. Bennett*³, *K. Roeder*⁴, *W.F. Byerley*¹. 1) Dept Psychiatry, Univ California, Irvine, CA; 2) Dept Psychiatry, Univ Pittsburgh, Pittsburgh, PA; 3) Dept Psychiatry, Univ Utah, Salt Lake City, UT; 4) Dept. Statistics, Carnegie Mellon University, Pittsburgh, PA.

To identify some of the genes underlying liability to schizophrenia, we have been studying large multiplex kindreds derived from the isolated populations of Daghestan, an republic located in southwestern Russia. The Daghestanian families reside in remote villages located in the Caucasus mountains. Due to substantial endogamy and inbreeding, many of these villages are essentially extended kindreds. Some of these kindreds have a substantial number of schizophrenics; others have none. As part of an ongoing study, we present the preliminary results from a linkage analysis for 2 large, multiplex kindreds, K3505 and K3506, based on a 10 cM genome screen (Weber/CHLC 9.0 markers) performed by the Mammalian Genotyping Service. Five affecteds and 12 unaffecteds/unknowns were genotyped for K3505, which is a complex pedigree of 142 individuals; 6 affecteds and 11 unaffected/unknown were genotyped for K3506, which is a complex pedigree of 109 individuals. Linkage analyses were performed using descent graph methods (Sobel and Lange 1996; *Am J Hum Genet* 58:1323-1337), as implemented in Simwalk2. In the first stage of our analysis, we obtained whole genome marker-sharing statistics. Due to sparse sampling and pedigree complexity, these non-parametric statistics were quite conservative, with most p-values close to 0.5. To identify promising regions, by kindred, we looked for statistics that were unusually large relative to the set of statistics obtained from across the genome. In the next stage, these promising regions were analyzed using parametric linkage with the choice of genetic model guided by the results obtained in the non-parametric analysis. Finally we genotyped a denser grid of markers in the most promising regions. While the analyses are ongoing, the most positive findings were 1q32 (LOD = 1.8), 2q24 (LOD = 2.3) and 17p11-12 (LOD = 2.1).

A genome wide search for dyslexia loci in a large Norwegian family. *T. Fagerheim¹, P. Raeymaekers², F-E. Toennessen³, L.A. Sandkuijl⁴, H.A. Lubs⁵, L. Tranebjaerg¹*. 1) Dept Medical Genetics, Univ Hosp Tromsø, Tromsø, Norway; 2) LyRaGen, Lint, Belgium; 3) Center For Reading Research, Stavanger, Norway; 4) Leiden University, The Netherlands; 5) Division of Genetics, Department of Pediatrics, Miami, FL, USA.

Increasing knowledge about the genetics of dyslexia has evolved from many studies during the recent years, and localization of genes for dyslexia has been reported on chromosomes 15 (DYX1), 6p21.3-23 (DYX2), and 1p. We have investigated a large Norwegian family in which dyslexia is inherited as an autosomal dominant trait, and we have previously reported the localization of DYX3 to 2p15-p16 (Fagerheim et al., 1999, JMG;36:664-669). We now report the complete genome wide search in this family with an average 15 cM marker density. Denser sets of markers were used in the regions previously reported to be implicated in dyslexia. The genetic analysis was performed in 36 of the 80 family members, and three different diagnostic models were used in the calculations in the linkage analysis. The linkage analysis was performed with both a standard lod score analysis and the non-parametric GENEHUNTER approach. We have excluded the possibility of an influence from DYX2 and the loci on 1p on dyslexia in this family. However, positive lod scores for markers at the DYX1 locus on chromosome 15 may suggest that a modifier locus is involved in the expression of the dyslexia phenotype in this family.

The effect of genotype error on the power to detect linkage and association with quantitative traits. *L.R. Cardon, G.R. Abecasis, S.S. Cherny.* Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, England.

Misclassification of genotypes can create severe problems for genetic mapping studies, such as inflation of map distances and confounding of marker order. Recently, Douglas et al (*Am J Hum Genet*, 66:1287-1297, 2000) extended these well-known results to the case of complex trait mapping, showing that relatively low genotype error rates (0.5% - 1%) can decrease the power of affected sib-pair mapping by as much as 50%. Given the low power of such linkage studies in the best case of no genotyping error, these results underscore the importance of genotype accuracy. Here we evaluate the effects of genotype error on linkage and association studies of continuous characters by simulation of randomly ascertained sibling pairs with no parental data.

Unlike affected sib-pairs, linkage analyses of quantitative traits generally do not suffer a dramatic loss in power due to genotype error. For standard identity by descent linkage mapping, less than 10% power is lost for error rates below 2%. With increasing error rates, however, the reduction of power can be substantial, as 10% genotyping error can reduce power by one-third. For unselected families, multipoint linkage results are not more strongly influenced by error than single marker analyses.

The effects of genotype error on the power to detect allelic association are more dependent upon allele frequency than error rate. While common alleles (minor allele frequency > 20%) are fairly robust to error in the realistic range (0.5% - 5%), the effects can be dramatic with less frequent alleles; e.g., misclassification of 1/50 calls of an allele having 5% frequency results in a 35% loss of power to detect the association. At 5% genotyping error, 55% of the power is lost. Given the imminent release of a SNP map of > 300,000 markers, these results may help guide selection of high throughput genotyping protocols and sample size.

Identity by descent and candidate gene mapping of Richieri-Costa- Pereira syndrome. *R.L.L. Ferreira de Lima*^{1,2}, *D. Moretti-Ferreira*^{2,3}, *A. Richieri-Costa*³, *M. Marazita*⁴, *J.C. Murray*¹. 1) Pediatrics, Univ Iowa, Iowa City, IA; 2) Departamento de Genetica, Universidade Estadual de Sao Paulo, Botucatu, SP, Brazil; 3) Servico de Genetica Clinica, Hospital de Anomalias Craniofaciais, Universidade de Sao Paulo, Bauru, SP, Brazil; 4) University of Pittsburg.

The Richieri-Costa-Pereira syndrome is a rare autosomal recessive disorder characterized by short stature, Robin Sequence, cleft mandible, pre/postaxial anomalies and clubfoot (Richieri- Costa and Pereira, 1992). The syndrome was described in the South of the State of Sao Paulo and has been seen almost exclusively in Brazil, suggesting this condition represents a rare mutation and founder effects (Richieri-Costa and Pereira, 1993). We have collected eleven affected (8 females and 3 males) from 10 apparently unrelated families with this unique syndrome. In the initial phase of research, DNA from 7 affected individuals were genotyped with Marshfield set 9 markers and compared to controls. To date we have typed these individuals with 115 markers and found no evidence for allele sharing. In a complementary analysis we are examining linkage and doing direct sequencing of exons for a group of candidate genes selected for their expression in limb and craniofacial structures. To date we have excluded PAX9, MSX1 and PITX1. This analysis holds out the prospect for using identity by descent approaches in a very manageable number of individuals from a specific geographic region to isolate mutations with unique phenotypes. In the next phase we will genotype the affected individual with the remaining 262 polymorphic markers and sequence the candidate genes DLX5/6, PITX2, TBX4, TBX5 and LIMH1.

Genome scan for psoriasis susceptibility genes in Newfoundland families suggests predisposing loci distinct from other populations. *P. Charmley*¹, *R. Leder*², *M. McEuen*¹, *B. Fessenden*¹, *E. Farber*², *W. Gulliver*³. 1) Celltech Chiroscience, Inc., Bothell, WA; 2) Psoriasis Research Institute, Palo Alto, CA; 3) Dermatology Research Center, St. John's, Newfoundland Canada.

Psoriasis is a skin disease which is characterized by hyperproliferating keratinocytes and infiltrating leukocytes, in which the disease onset is associated with environmental factors and also multiple susceptibility genes. A variety of groups have published studies reporting the possible locations of several psoriasis susceptibility loci, including regions on chromosomes 1q, 3q, 4q, 6p, 16q, 17q, and 20p. In order to test these reported localizations, we have gathered a collection of families with members affected with psoriasis from a relatively homogeneous population from the eastern-most Canadian province of Newfoundland. DNA from a group of 103 families was collected in which each family had between 2-6 affected siblings diagnosed with plaque-type psoriasis. The segregation of these chromosomal regions was studied using >100 microsatellite polymorphisms. Of all these chromosomal regions, the chromosome 6p region containing the HLA loci showed the strongest support for containing a susceptibility allele, based on an NPL score of about 2.9 ($p=0.002$). Based on an association of HLA Cw6 with psoriasis in these families ($p<10E-12$), the susceptibility allele in this chromosome 6p region will be found on a similar HLA Cw6 haplotype as previously published by other groups. In contrast, this collection of Newfoundland families did not offer support for any of the other listed chromosome regions ($p>0.05$). We conclude that there will be some overlap in psoriasis susceptibility alleles between this collection of families from Newfoundland and other published family collections (e.g. in the HLA region). However, for most of the other chromosomal regions previously published by other groups, these Newfoundland families will not offer statistical support for the existence of predisposing loci in those regions.

Predominance of renal dysfunction in male SLE patients and their relatives and tentative linkage to chromosome

15. *C.M. Jedrey¹, J.M. Olson¹, C. Gray-McGuire^{1,2}, J.B. Harley², K.L. Moser^{1,2}.* 1) Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH; 2) Oklahoma Medical Research Foundation, Oklahoma City, OK.

Systemic lupus erythematosus (SLE) is a complex autoimmune disorder of unknown etiology. Clinical identification of genetically homogeneous subsets of SLE families would greatly facilitate gene identification. Only 5-10% of SLE patients are male and higher prevalence of renal dysfunction (RN) in male lupus patients is commonly observed. However, rates of RN in affected female relatives of affected males have not been reported previously. We present results from 160 pedigrees multiplex for SLE, including 25 with at least one affected male. These 25 families include 15 European American (EA), 7 African American, 2 Hispanic, and 1 Native American families. Initial analysis of clinical features in 126 pedigrees, 19 of which had affected males suggested that increased presence of RN in males is familial rather than a gender difference. Addition of 34 pedigrees, 6 of which included affected males, confirmed these results; the proportion of affected males ($n=27$) and their affected female relatives ($n=38$) with RN was 56% and 68%, respectively, compared to 43% in affected females without affected male relatives ($n=307$) ($p=0.003$). After stratifying by race, this trend remained, but was more pronounced in the EA pedigrees. A similar familial difference existed for hematological dysfunction; 78% of males and 84% of their female relatives met this criteria compared to 67% of females without male affected relatives ($p=0.015$). A genome scan using conditional logistic affected pair method showed tentative evidence for linkage on chromosome 15 ($\text{lod}=1.70$) in the 25 pedigrees, versus no evidence for linkage in the remaining families ($\text{lod}=0$). When examining just the 15 EA families with affected males, the lod score increased to 1.97. These data suggest that presence of renal disease in male lupus patients reflects a familial rather than a gender difference, particularly in European Americans, a first step toward clinical identification of genetically distinct SLE subsets.

Fine-mapping and Genetic analyses of Sitosterolemia: Founder effects in at least three geographic areas. *M.H. Lee¹, K. Lu¹, D. Gordon⁶, J. Ott⁶, H. Kojima², P.O. Kwiterowich⁷, M.J. Brownstein⁸, G. Salen³, L. Ose⁹, T. Mietinnen⁴, R. Pegoraro⁵, H. Hidaka¹⁰, N. Sakuma¹¹, A. Pandya¹², S. Patel¹.* 1) Medical Univ South Carolina, Charleston, SC; 2) Shiga University of Medical Science, Shiga, Japan; 3) UMDNJ, New Jersey, NJ; 4) Helsinki University, Helsinki, Finland; 5) University of Natal, Durban, South Africa; 6) Rockefeller University, New York, NY; 7) Johns Hopkins University, Baltimore, MD; 8) NIMH/NHGRI Bethesda, MD; 9) Rikshospitalet, Oslo, Norway; 10) Sanyo, Tokyo, Japan; 11) Third Dept Medicine, Nagoya City University, Nagoya, Japan; 12) Dept of Human Genetics, Medical College of Virginia, Richmond VA.

Sitosterolemia is an autosomal recessive disorder (OMIM 210250) that results in the abnormal accumulation of xenobiotic sterols in the plasma and tissues in humans. There is increased net dietary absorption and retention of xenobiotic sterols, such as sitosterol. Affected individuals are at increased risk of premature atherosclerosis and cardiac deaths in affected teenagers have been reported. We have previously mapped the gene defect to human chromosome 2p21 to a 15 cM interval. Here we report the fine mapping and genetic analyses in over 28 families, narrowing the interval to less than 2 Mb, with a maximum multipoint lodscore of 10 for microsatellite marker D2S119. By haplotype analyses, homozygosity was detected in many of the probands. Interestingly, homozygosity and haplotype-sharing was found for probands from Finland, Norway and Sweden, suggesting a founder effect for the Scandinavian probands. A founder effect amongst the Amish was also detected, and showed that two previously 'unrelated' families were closely related, and the gene in the Amish population may have been introduced into the American families by the first Amish settlers. Finally homozygosity and haplotype sharing was also found in some but not all of the Japanese probands, suggesting another possible founder effect. A sequence-ready BAC and a transcript map contig was constructed of this region to help facilitate the identification of the defective gene.

Clinical and genetic study of a large Charcot-Marie-Tooth type 2A family from Southern Italy. *M. Muglia¹, M. Zappia², V. Timmerman⁵, P. Valentino², AL. Gabriele¹, F.L. Conforti¹, P. De Jonghe⁵, M. Ragno³, R. Mazzei¹, M. Sabatelli⁴, G. Nicoletti¹, AM. Patitucci¹, RL. Oliveri^{1,2}, F. Bono², A. Gambardella^{1,2}, A. Quattrone^{1,2}.* 1) Institute of Experimental Medicine and Biotechnology, National Research Council, Mangone, Cosenza, Italy; 2) Institute of Neurology, School of Medicine, Catanzaro, Italy; 3) Institute of Neurology, Hospital G.C. Mazzoni, Ascoli Piceno, Italy; 4) Institute of Neurology, Catholic University, Rome, Italy; 5) Flanders Interuniversity Institute for Biotechnology, Born-Bunge Foundation, University of Antwerpen, Antwerpen, Belgium.

Charcot-Marie-Tooth type2 (CMT2) is a heterogeneous group of axonal neuropathies with normal or slightly low nerve conduction velocities, also usually inherited as autosomal traits. Linkage analysis studies have identified three genetically different varieties on chromosome 1p35-p36 (CMT2A), chromosome 3q13-q22 (CMT2B), and chromosome 7p14 (CMT2D). Moreover, there is a fourth CMT2 type (CMT2C) not linked to any known locus. Interestingly, distinct point mutations in the myelin protein zero (MPZ) gene may also be responsible for a CMT2-like phenotype. Until now, significant evidence for linkage to chromosome 1p35-p36 was reported for only five CMT2A families, from Japan or North America, making it difficult to assess the geographic distribution and clinical features of this disorder. In this study, we report the clinical and molecular genetic study in a large Italian family with CMT2A. The clinical picture was uniform and characterized by distal muscular weakness and atrophy in the lower limbs, reduced or absent tendon reflexes mainly in the lower limbs, and mild sensory impairment in the feet. A nerve biopsy showed decrease of myelinated fibers without active myelination. The age at onset was variable and there is some evidence that the youngest generation had an earlier onset. Significant linkage to the CMT2A locus on chromosome 1p35-p36 was detected. Based on informative recombinants, we mapped the CMT2A gene between D1S503 and D1S228 and refined the CMT2A region to approximately 10 cM.

Mapping genes for nephropathy in IDDM: Linkage analyses of eight typed chromosomes (2, 4, 6, 10, 12, 16, 20 and 22). *B. He¹, L. Liu¹, A-M. Osterholm¹, T. Berg¹, J. Tuomilehto², J. Pitkaniemi², J. Rogus³, A. Krolewski³, K. Tryggvason¹.* 1) MBB, Matrix Biology, Karolinska Institute, Stockholm, Sweden; 2) National Laboratory of Public Health, Helsinki, Finland; 3) Joslin Diabetes Center, Harvard Medical School, Boston, Massachusetts, USA.

Diabetic nephropathy (DN) is one of the major late kidney complications of diabetes. It is characterized by thickening of the glomerular basement membrane and clinically, it is manifested as persistent proteinuria. Genetic components might be involved in the development of DN, since only 30-35% of patients with type 1 diabetes (IDDM) are predisposed to nephropathy. The incidence of nephropathy peaks during the second decade of diabetes and declines thereafter. Mechanisms behind the pathogenesis of DN, however, are largely unknown. Here, we carried out a strategy of genome-wide scan to search for the gene(s) contributing to nephropathy susceptibility in IDDM patients. To map the genes, we have used a method of discordant sibpair (DSP) for linkage analysis. As a strategy, 95 DSP from US were typed as an initial scanning using 516 markers with an average spacing of 7 cM. Additional 45 Finnish DSP have been used for follow-up studies. A model-free linkage analysis was performed for all typing data. So far we have genotyped eight chromosomes (2, 4, 6, 10, 12, 16, 20 and 22). Multipoint analyses of chromosome 4, 6, 10, 12, 16 and 22 have excluded linkage to DN, when maximum lod score (MLS) above 1.0 is used as a hit level for follow-up studies. Two markers, one on chromosome 2p and another on 20p, resulted in MLS of 2.1 and 1.01 respectively. Interestingly, the hit of chromosome 20p overlapped results from a study of Pima Indian DN. In follow-up studies, 9 additional markers on chromosome 2p were typed in both the US and Finnish DSPs. The MLS increased to 2.5 in US families. However, this finding failed to be replicated in Finnish families (MLS = 0.56). Combined data of two populations resulted in a reduced MLS. Thus the locus of the chromosome 2p is unlikely to have a major role in DN, with possible exception of a particular population with minor effect. Analysis of saturated markers on chromosome 20p region is ongoing.

Locus heterogeneity in Knobloch syndrome. *O. Menzel*¹, *S. Aftimos*², *C. Gehrig*¹, *S.E. Antonarakis*¹, *H.S. Scott*³, *M. Guipponi*¹. 1) Medical genetics, University of Geneva, CMU, Geneva, Switzerland; 2) Northern Regional Genetics Service, Department of Clinical Genetics, Auckland, New Zealand; 3) Genetics and Bioinformatics Group, The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, Australia.

Knobloch syndrome (KNO) is a rare autosomal recessive disorder characterized by severe myopia, vitreoretinal degeneration with retinal detachment and occipital encephalocele. The clinical phenotype may also include lens subluxation, cataracts, tilted optic disc, epicanthal folds, flat nasal bridge, midface hypoplasia, and mild generalized hyperextensibility of the joints. One KNO locus was assigned to chromosome 21q22.3 between D21S171 and D21S1446 on the basis of homozygosity mapping in a large consanguineous Brazilian family. Here, we report the exclusion of the chromosome 21q22.3 candidate region in a small New-Zealand family. Haplotypes spanning the D21S171-D21S1446 region were reconstructed, both affected brothers were heterozygous and have inherited the same allele from their father but different alleles from their mother suggesting that the gene responsible of the KS in this family does not map between D21S171-D21S1446. Given that the microsatellite markers used, were closely spaced and flanked the candidate region on 21q22.3, the occurrence of a double crossing-over in one of the affected brother is highly unlikely. Thus, the present study provides evidence for the existence of a second KNO locus. We propose then to refer to the locus on chromosome 21q22.3 as KNO1 and the second locus as KNO2.

QTL localization accuracy of variance component linkage analysis methods. *D.L. Koller, B.P. McEvoy, T. Foroud.*
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Several linkage analysis packages implementing variance components models for quantitative traits are currently available. To compare the localization accuracy of several of these programs, we used GASP (Wilson et al, AJHG 59:A193, 1996) to simulate an 80 cM chromosome with markers of 80% heterozygosity 10 cM apart. We allowed two parameters to vary which are generally beyond the investigator's control: QTL effect size (10 and 25%) and overall trait heritability (50 and 80%). The remaining parameters which we varied are generally limited by the cost of the study: number of sibships studied (400 and 1000), QTL position (0, 2, or 5cM from nearest marker), and the number of parents genotyped (0,1, or 2). We analyzed 1000 replicates for each combination of parameter values with Mapmaker/SIBS (MM/S; weighted and unweighted) and Sibpal2 (beta 3) from the S.A.G.E. package.

We compared localization ability under the various conditions by calculating the mean distance (cM) between the position of the maximum LOD score and the true location of the gene for each set of simulation conditions. Overall, the mean localization performance was similar for all programs tested. We observed surprisingly large mean peak-to-gene distances even under the most ideal conditions. In the 1000-family sample, this distance ranged from 3.5 to 10 cM with all programs for a QTL of 25 percent effect, and from 12.5 to 16 cM with a locus effect of 10%. In the smaller (400-family) sample, the mean peak-to-gene distance ranged from 8 to 14 cM with a QTL of 25% effect, and from 16 to 18 cM for a locus effect of 10%. Interestingly, for a given QTL effect size, the ability of Mapmaker/SIBS to accurately localize QTLs depended primarily on the overall heritability of the quantitative trait. In contrast, SAGE/Sibpal2's localization ability was dependent primarily on the proximity of the QTL to a marker locus, and overall trait heritability was of little importance. We conclude that 10 cM genome screens will provide only very rough QTL localization, and follow-up genotyping of additional markers will be necessary.

Localization of the gene for Distal Hereditary Motor Neuronopathy-VII to chromosome 2. *M.E. McEntagart¹, N. Norton², H. Williams², H. Houlden³, E. Boltshauser⁴, M. Donaghy⁵, P.S. Harper¹, N. Williams², N. Rahman¹.* 1) Institute of Medical Genetics, UWCM, Cardiff, Wales, UK; 2) Department of Psychological Medicine, UWCM, Cardiff, Wales, UK; 3) Neurogenetics, Institute of Neurology, Queen Sq, London, UK; 4) Department of Paediatrics, University of Zurich, Switzerland; 5) Department of Clinical Neurology, Radcliffe Infirmary, Oxford, UK.

The distal Hereditary Motor Neuronopathies (dHMN) are characterised by distal muscular atrophy and weakness similar to that seen in Hereditary Motor and Sensory Neuropathy types I and II (HMSN I and II, also known as CMT I and II). However, in contrast to HMSN, there is no sensory involvement in the hereditary motor neuronopathies. Several genes and many loci that confer susceptibility to both groups of conditions have been identified. dHMN-VII is an autosomal dominant condition presenting with progressive distal muscle wasting in association with vocal cord paralysis. Electrophysiology studies suggest that the disease process occurs at the level of the anterior horn cell, and thus the condition is also known as distal spinal muscular atrophy-VII. We have investigated a large Welsh family that includes multiple individuals with distal muscular atrophy in association with either unilateral or bilateral vocal cord paralysis (dHMN-VII). We excluded linkage to the candidate gene, *MPD2*, and have subsequently performed a genome-wide linkage search. This has localised the gene for dHMN-VII to a region of chromosome 2 (two-point LOD score 3.77) that has not been previously implicated in either HMN or HMSN disorders. We are now evaluating other families with similar phenotypes for confirmatory evidence of the gene location and to assess whether there is heterogeneity in the predisposition to distal muscular atrophy associated with vocal cord paralysis.

Genome-wide scan for Paget's disease of bone in French-Canadian families. *N. Laurin¹, V. Raymond¹, A. Duchesne¹, C. Brousseau², D. Huot², Y. Lacourcière², G. Drapeau², J. Verreault², J.P. Brown², J. Morissette¹.* 1) Molecular Endocrinology and Oncology, CHUL (Laval University Medical Ctr) Research Ctr, Quebec city, Qc., Canada; 2) Rheumatology-Immunology, CHUL Research Ctr, Quebec city, Qc., Canada.

Paget's disease of bone is a chronic skeletal disorder characterized by an excessive increase of bone turnover. The pathologic basis of the disease is still unknown. Recent pedigree studies have shown that the modes of genetic transmission of Paget's disease of bone include both autosomal dominant inheritance with reduced penetrance and multifactorial inheritance. To understand the genetic components underlying the disorder, we performed a genome-wide scan on three family nuclei selected from 24 French-Canadian kindreds in which Paget's was transmitted as an autosomal dominant trait. These nuclei comprised 27 affected and 17 non-affected subjects. Diagnoses were based on total serum alkaline phosphatase, total body bone scan and skull and pelvis X-rays. We genotyped 400 microsatellite markers located at an average spacing of 10 cM. Linkage analyses were done by FASTLINK 4.1P and GENEHUNTER 2.0. Haplotypes were phased by SIMWALK 2.6. With a phenocopy rate fixed at 5% for people up to 50 years old, the highest lod score values were observed at D19S414 ($Z=3.03$), D3S3681 ($Z=1.37$), D15S153 ($Z=1.27$), D4S422 ($Z=1.22$) and D1S2890 ($Z=1.16$). Heterogeneity was observed for several markers between the families. When families were analyzed separately, a few chromosome regions showed positive lod score values between 0.75 and 2.00 at independent loci. Multipoint analyses were subsequently performed within regions of interest. Some consecutive markers (between 3 to 7) gave positive lod score values on chromosomes 3, 4, 9, 10, 13, 16, 19 and 20 in one or more families. These data therefore suggest that Paget's disease of bone may be genetically heterogeneous. The seven most interesting regions are now being investigated for linkage with the disease using additional markers and the 21 other families not included in our genome-wide scan.

Sampling considerations for family-based tests of association. *E.R. Martin¹, S.W. Hardy², M.P. Bass¹, N.L. Kaplan³.*

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Family-based tests for association are typically proposed for nuclear family or sibship data. Recently, the pedigree disequilibrium test (PDT) has been proposed to test for association in extended families (Martin et al. 2000, Am J Hum Genet). The PDT has been shown to be a powerful alternative to existing methods, even in nuclear families. Thus, it provides a general test for association that can be applied in families of different pedigree structure. The PDT was proposed to provide a method to take advantage of data in large pedigrees that may be collected for other analyses, but optimal family size for the PDT has not been addressed. When designing a study aimed at association analysis, it is desirable to know whether resources should be invested in obtaining a large number of small nuclear families or sibships, or whether they are better directed toward collecting fewer large families. Using computer simulation, we compare the power of the PDT in families of different size and structure, holding the total number of individuals constant. A range of genetic models is considered to study the impact of model parameters on relative power. Our results suggest that, for many complex diseases, the power may be similar for families of different size with a fixed number of individuals in the sample, but specific design recommendations will be dependent on the genetic model and family structure. Factors such as ease in ascertainment, disease prevalence, age of onset, and other types of analyses to be conducted may be most important in determining the desirable family size and structure for association studies of complex diseases.

Genetic mapping of families with congenital nystagmus (CN). *W.S. Oetting¹, C. Armstrong¹, A. Holleschau², A.T. DeWan³, R.A King¹, C.G Summers².* 1) Dept Med, Gen/Box 485, Mayo, Univ Minnesota, Minneapolis, MN; 2) Dept Ophthalmology, Univ Minnesota, Minneapolis, MN; 3) Dept Epidemiology, Univ Minnesota, Minneapolis, MN.

Congenital nystagmus (CN) is a relatively common genetic disorder (approximately 1 in 1,500) characterized by bilateral involuntary ocular oscillations with onset typically occurring within the first 6 months of life. Reported patterns of inheritance for CN include autosomal dominant, autosomal recessive and X-linked. To date, three loci associated with CN have been mapped to chromosomes 6p12, Xp11.4-p11.3 and Xq26-q27. We have analyzed 5 pedigrees segregating for CN. Families were recruited when a patient 1) presented with conjugate nystagmus that had been present since the first few months of life, 2) had no evidence of other central or ocular disease, and 3) exhibited a family history containing at least two generations of affected individuals. Of those families analyzed, all pedigrees included multiple generations of affected individuals. Mapping studies using markers in those regions that previously exhibited linkage for CN were used. Linkage analysis was done using LINKAGE for two point analysis and GENEHUNTER 2 for multipoint analysis. Linkage analysis showed that only one pedigree exhibited suggestive linkage with a lod score of 2.08, $q = 0.0$, at chromosome Xp11. This pedigree has both affected male and female members, with two unaffected obligate female carriers. The remaining 4 pedigrees did not exhibit evidence of linkage for any of the three chromosome locations. Three pedigrees, Pedigrees 2, 4 and 5, exhibited several instances of male-to-male transmission, excluding X-linkage, and exhibited a lod score of -3.64, $q = 0.0$ for marker D6S459 located at 6p12, thus excluding the chromosome 6 locus. This provides evidence for at least a fourth locus associated with CN.

Molecular analysis of an Australian family with late onset dominant hearing loss. *M. Kamarinos, McR.J. Gardner, H.-H.M. Dahl.* The Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, Australia.

We have recruited an Australian family presenting with non-syndromic late onset progressive hearing loss that is inherited in a dominant fashion. The hearing loss presents in the second to third decade of life with the onset becoming earlier and the decline more rapid with each successive generation. There is evidence of vestibular involvement, including episodes of vertigo in affected family members. The progressive deafness is associated with a progressive and extensive loss of vestibular function. The concomitant loss of vestibular function is present in 10 per cent of cases of progressive hearing loss making this family an interesting one for genetic analysis. Our aim is to identify the gene causing hearing loss in this family using a traditional mapping approach. We have DNA samples from 8 affected and 5 unaffected family members. Linkage analysis on the known dominant deafness loci showed no evidence of linkage suggesting that the gene causing hearing loss in this family is novel. SLINK analysis of this family yielded a maximum LOD score of 3.08. We then undertook a 10 cM whole genome wide scan using 381 polymorphic markers. MLINK analysis of the data revealed 9 loci with LOD scores above 1.0. DNA from an additional 6 affected family members have now been obtained and are being used to further fine map these 9 loci to find which one encompasses the new hearing loss gene.

A New Gene For Autosomal Dominant Nonsyndromic Sensorineural Hearing Loss (DFNA32) Maps to 11p15.

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Hearing loss is the most common sensory defect in humans. About one in every 1000 children is affected by severe hearing loss at birth or during early childhood. More than 50% of these cases are due to genetic cause with extensive genetic heterogeneity. A genetic linkage study conducted on a large multigenerational US family with nonsyndromic autosomal dominant progressive hearing loss has resulted in the localization of a new deafness locus, DFNA32. The deafness gene segregating in this family is mapped to the telomere region of chromosome 11p15. A maximum lod score of 4.1 was obtained with Marker D11S1984. The DFNB18 and two syndromic deafness genes, Usher syndrome type 1C and Jervell and Lange-Nielson syndrome have been mapped to the proximity of this interval. The DFNA32 interval is approximately 3-4 cM distal to DFNB18 and Usher 1C, but does not overlap with them.

Discordant age of diagnosis in Type 1 diabetes mellitus provides evidence for linkage to the *IL2* region. A.D. Paterson. Dept Genetics, The Hospital for Sick Children, Toronto, Ontario, Canada.

Although there is wide variation in the age of diagnosis of type 1 diabetes mellitus (insulin dependent diabetes), few genetic studies (with the exception of HLA) have incorporated this information. We hypothesized that relative discordance of age of diagnosis may assist in the detection of novel loci linked to type 1 diabetes, in a similar way to which *ADH3* on chromosome 4q21-q23 shows the most significant linkage to sibpairs discordant for alcoholism. To test this we used data from a genome scan of 356 Caucasian families with sibpairs affected with type 1 diabetes from the UK (Mein et al., 1998). The mean and median age of diagnosis in these families was 10 years of age. We thus selected only those families (n=131) where one sib was diagnosed earlier than 10 years of age, and the other sib was diagnosed later than 10 years of age. We tested for excess non-sharing of alleles using data from 351 autosomal markers from across the genome. The most significant excess non-sharing of alleles was observed at marker D4S430 (chromosome 4q26-q27) where there was sharing of 56 parental alleles identical by descent compared to non-sharing of 111 parental alleles. The proportion non-sharing was 34% ($c^2=18.1$, 1 df, $p = 2.1 \times 10^{-5}$). This result just meets the Lander and Kruglyak criteria for significant linkage. In contrast there was no significant excess non-sharing of alleles in sibpairs where both were diagnosed under (n=130), or over (n=95) the age of 10 years. The linkage of families with discordant ages of diagnosis of diabetes to D4S430 is of interest since approximately 2 cM from this marker is the interleukin 2 gene (*IL2*). In the NOD mouse model of diabetes there is growing evidence that *IL2* is the gene on murine chromosome 3 underlying the susceptibility locus termed *Idd3*. Attempts to replicate and fine-map the linkage to human chromosome 4q26-q27 using additional families that are discordant for age of diagnosis are necessary. Also, clarification of the most appropriate age of diagnosis criteria at this locus is warranted, as are association studies of polymorphisms in *IL2*.

Linkage of benign familial infantile convulsions to the ICCA region on chromosome 16. *P. Szepietowski¹, S. Pavsek², R. Caraballo³, M. Gastaldi¹, A. Faure², A.P. Monaco⁴, N. Fejerman³, J. Rochette⁵, A. Lemainque², G.M. Lathrop².* 1) Inserm U491, Faculte de Medecine La Timone, Marseille, France; 2) Centre National de Genotypage, Evry, France; 3) Hospital J Garrahan, Buenos Aires, Argentina; 4) The Wellcome Trust Centre, Oxford, UK; 5) Genetique Medicale, Amiens, France.

The ICCA syndrome is characterised by the variable association of benign infantile convulsions (BFIC) with paroxysmal dyskinesias, and an autosomal dominant mode of inheritance. The disease had been linked to chromosome 16p12-q12 in several unrelated families. Families with either BFIC or paroxysmal dyskinesias have also been described. Paroxysmal dyskinesias of the kinesigenic type (PKD) recently have been linked to chromosome 16p12-q12 as well, suggesting that the ICCA syndrome and PKD could be allelic. On the other hand, pure BFIC had been linked to chromosome 19q, while data on additional families argued in favor of genetic heterogeneity. We have collected several families from France and Argentina, in which BFIC was inherited as an autosomal dominant trait. No dyskinesia was diagnosed in any of the patients prior to the analysis. Linkage analysis has been performed with polymorphic markers at chromosomes 16p12-q12 and 19q. Preliminary data suggest exclusion of chromosome 19q while linkage with markers situated within the ICCA critical region at 16p12-q12 is strongly suspected (two-point LOD score = 2.8). Additional affected members are currently genotyped and should help reach more significant LOD scores. These results suggest that mutations of a single gene at chromosome 16 could lead to either benign convulsions, or paroxysmal dyskinesias, or both (ICCA). Moreover, one patient in the BFIC families is homozygous for the disease haplotype and did not respond well to anticonvulsants, while all other patients responded correctly. This patient also started displaying abnormal movements at age 2, which was not the case for any other member of the family. These findings could provide interesting clues to understand the pathogenetic mechanisms of BFIC as well as the molecular basis of the variable expressivity of the ICCA phenotypes.

Evidence for yet another novel Bardet-Biedl Syndrome (BBS) locus in the Newfoundland population. M.O.

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The island of Newfoundland, off the northeast coast of Canada, has had a relatively isolated population for more than 200 years. Made up primarily of English, Irish and Scottish ancestry it has been considered a homogenous populace, and until very recently, distributed mainly in small remote coastal villages. Such a population is ideal in investigating rare recessive conditions.

One such disorder is Bardet-Biedl Syndrome (BBS), which is ten time more prevalent in Newfoundland than in northern European populations. BBS is characterized by retinal dystrophy, renal anomalies, dysmorphic extremities, obesity and hypogenitilism in males. Five autosomal loci have been identified: BBS1(11q), BBS2(16q), BBS3(3p), BBS4(15q) and BBS5(2q); three of which (BBS1,BBS3,BBS5) are known to segregate in the Newfoundland population. Both the BBS1 and BBS3 loci were identified in Newfoundland by a population survey of the first four loci using 17 BBS families and the latter via a genome wide scan, implementing homozygosity mapping, in a large consanguineous kindred.

Another consanguineous BBS family could be excluded at all five known BBS loci, providing evidence for yet another BBS locus in our population. A genome wide scan, utilizing homozygosity mapping, was undertaken to identify this novel BBS locus. After one-third of the genome scan was completed a 3:1 shift in alleles from the control pools, containing DNA from parents and unaffected sibs, to the test pool, from two affected individuals, was observed with a microsatellite marker on 20p. Genotyping of the entire family revealed that only the affected patients were homozygotes and this homozygosity was extended to a 16cM region, while all unaffected individuals were heterozygotes. Due to the structure of this family a significant lod score could not be obtained. Therefore, other families, unlinked to all five loci, were analyzed with the relevant markers and additional kindreds were found that were consistent with 20p containing a new BBS locus.

Refining the candidate region for schwannomatosis. *C.J. Willett¹, L.B. Jacoby¹, D. Kaufman², M. MacCollin¹.* 1) Neurology and Neurosurgery, Massachusetts General Hospital, Charlestown, MA; 2) Surgery, Stanford University, Stanford, CA.

In our previous work, we have shown that schwannomatosis is a third form of NF which is associated with unique genetic patterns, natural history and molecular biology. Because of the patterns of LOH observed in schwannomatosis associated tumors, we have hypothesized that the schwannomatosis genetic change may be a tumor suppressor which is inactivated through a contiguous sequence loss of material with *NF2*.

In the current study, we have refined the candidate region for schwannomatosis on chromosome 22 using family studies and loss of heterozygosity analysis. We have identified 7 families in which schwannomatosis is segregating in an autosomal dominant fashion with incomplete penetrance and confirmed somatic instability of *NF2* (multiple tumors with different small inactivating mutations with LOH of the allele trans to the shared chromosome) in 18 tumors from 5 of the 7 families. Linkage analysis using markers in the *NF2* region now yields a maximum LOD score of 3.33 at $q=0.0$. A recombination event involving an affected individual places schwannomatosis proximal to a newly developed marker which is approximately 150kB distal to *NF2*. In parallel studies, 22 schwannomatosis derived tumors with known LOH at the *NF2* locus were compared to paired blood specimens at microsatellite markers spaced on the long arm of chromosome 22. All 22 tumors showed loss at proximal markers, while 3 tumors from a single patient showed retention at one or more distal markers. The most proximal retained marker was D22S430.

These two lines of study suggest that the schwannomatosis genetic change lies proximal to D22S268/430. Current work is focused on further refining the candidate region, examination of potential candidate genes, and further study of non coding regions of the *NF2* gene itself.

Refinement of a Locus on Chromosome 16q24.3 for Lymphedema-Distichiasis. *D.A. Persaud, S.A. Schmidt, S.G. West, M.A. Pericak-Vance, J.M. Vance, M.T. McDonald.* Duke University Medical Center, Durham, NC.

Lymphedema with Distichiasis (LD) (MIM153400) is an autosomal dominant disorder characterized by congenital distichiasis (an aberrant second row of eyelashes arising from or near the meibomian gland orifices) and primary lymphedema usually evident around the time of puberty. Evidence for linkage of a gene responsible for LD to chromosome 16q24.3 has been established previously by Mangion et al., (1999). We genotyped individuals from a three-generation North American pedigree using markers from this region to determine if this family exhibited linkage to 16q24.3 and to attempt to refine the localization. Individuals were characterized as 'affected' if they exhibited distichiasis on examination. Of the thirteen affected individuals, 9 had lymphedema, 4 had neck webbing, 2 had ectropion, and 1 had a cleft palate, demonstrating the clinical variability of LD. We report the genotyping results and analysis of 8 markers over an approximately 16cM range for this family. The statistical analyses were carried out using the VITESSE and SIMWALK2 programs. A maximum two point LOD score of 5.20 was obtained for the marker D16S498. A maximum multipoint LOD score of 5.99 indicates that the most likely location of the disease locus is between the markers D16S520 and D16S498. This is supported by critical recombination events from haplotype analysis. Our study confirms the linkage of a disease locus for Lymphedema Distichiasis to chromosome 16q24.3 and narrows down the interval likely to contain this locus from 16cM to 2cM.

The genealogical approach to complex genetic diseases. *K. Stefansson, A. Kong, J.R. Gulcher.* DeCode Genetics, Inc, Reykjavik, Iceland.

We describe a unique approach, the genealogical approach, in which the phenotype is defined broadly, but rigorously, and an extensive computerized genealogy database is used to determine which components of the phenotype are passed between generations in large extended families. When presented with the challenge of finding the genetic factors contributing to a complex phenotype, one can attempt to split and subclassify the patients. Unfortunately, most clinical phenotypes are based on consensus criteria that may be useful for clinical description but may not represent the traits that are inherited. While there is likely to be more than one genetic cause for common diseases, even in the most isolated of populations, we postulated that clustering patients using genealogy would further decrease the genetic complexity with respect to the patients within the cluster. Thus, this genealogical approach would have the effect of further simplifying the genetic makeup of patients analyzed for genome segment sharing. Note this represents an approach distinct from what is most often suggested in the complex genetic diseases; that is, fractionation of the patient material according to phenotypic subtypes in the hope of decreasing the genetic complexity. Fractionation of clinical material has the side effect of decreasing the number of individuals studied, potentially decreasing power. Furthermore, the approach of narrowing the phenotype defies some of the experience acquired in the study of the Mendelian diseases which often show variable expressivity. This approach was used to study the genetics of stroke, osteoarthritis, and Alzheimers disease, each broadly defined as a public health problem. In each case this approach successfully mapped at least one disease gene meeting the criteria of genome-wide significance. The genealogical approach is one way to: 1) deal with the elusive phenotype in some complex genetic diseases, 2) decrease the impact of locus heterogeneity, and 3) increase the genetic power by bringing together more distantly related patients and creating larger family clusters.

A locus for a severe form of X-linked myopia maps to the pseudoautosomal region of Xq28. *U. Radhakrishna¹, R. Raval², M.A. Morris³, A. Paoloni-Giacobino¹, J.-L. Blouin³, S. Raminder⁴, A.R. Vasavada⁴, J.V. Solanki⁵, S.E. Antonarakis^{1,3}.* 1) Dept Medical Genetics, Univ Medical School, Geneva, Switzerland; 2) Department of Biochemistry, Gujarat Cancer and Research Institute, Civil Hospital, Ahmedabad, India; 3) Cantonal Hospital, Geneva, Switzerland; 4) Iladevi Cataract and IOL Research Centre, Raghudeep Eye Clinic, Ahmedabad, India; 5) Veterinary College, Gujarat Agriculture University, Anand, India.

The phenotype of myopia is partly influenced by hereditary factors. Myopia may occur as an isolated genetic anomaly or it may be associated with other anomalies and syndromes. We have studied a large six generation Indian pedigree with isolated, non-syndromic myopia, in which the anomaly appeared to segregate as an X-linked recessive trait (OMIM 310460). The anomaly was only present in male members of the pedigree. The degree of myopia was variable ranged from -6 to -16.5 D with a mean of -13.33 D. Fundus examination revealed myopic degeneration of all the examined affected patients and the average anterior chamber depth was 3.28 mm. In order to map the myopia locus in this family, we have performed linkage analysis on 26 individuals including 9 affected and 17 normals, using polymorphic microsatellite markers covering the entire X chromosome. Marker DXYS154 which is located in pseudoautosomal region in distal Xq28, showed no recombination with the phenotype with a maximum LOD score of 3.99 at $\theta = 0$ under an autosomal recessive model. Other markers in the region (near but not within the pseudoautosomal region) that showed no recombination with the phenotype included DXS1108, DXS8087 and F8i13. This represents the first localisation of the gene responsible for severe form of myopia to the pseudoautosomal region of Xq28 that spans less than 500 kb. These results provide the basis for the identification of candidate genes responsible for severe myopia.

X chromosome statistics for nonparametric linkage analysis on general pedigrees. *K.K. Song, D.E. Weeks, E. Feingold.* Human Genetics, University of Pittsburgh, Pittsburgh, PA.

The literature on nonparametric linkage analysis with general pedigrees has been remarkably silent on the question of testing linkage to the X chromosome. Both Allegro and older versions of Genhunter have implemented X chromosome versions of the popular statistics S_{all} and S_{pairs} , but there is no published description of how these statistics are calculated, let alone any comparison of their power. The two programs have identical definitions of S_{all} , but they do not use the same version of S_{pairs} . The definition of S_{pairs} implemented in Allegro matches the definition implemented in Genhunter version 1.1, but Genhunter version 1.3 uses a different definition. We have compared the power of these three statistics to detect genes under several different simple and complex genetic models. We also consider the question of whether there exists a statistic that is better than any of these three choices.

Molecular analysis of Facioscapulohumeral Muscular Dystrophy (FSHD) in the South African Population. *A. van der Merwe¹, C-M. Schutte², S.M. van der Maarel³, E. Honey¹, R.R. Frants³, A. Olckers¹.* 1) Human Genetics, University of Pretoria, Pretoria, South Africa; 2) Neurology, University of Pretoria, Pretoria, South Africa; 3) Human Genetics, Leiden University Medical Centre, The Netherlands.

This study represents the first attempt to characterise the FSHD locus within the South African population. Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant neuromuscular disorder. It is characterised at onset by the weakness of the facial and shoulder girdle muscles, generally in the second decade of life. The gene for FSHD has not yet been identified, but linkage was established between the FSHD phenotype and short tandem repeat polymorphism (STRP) alleles in the sub-telomeric region of chromosome 4q35. It is currently estimated that at least 5% of FSHD families do not display linkage to chromosome 4, indicating genetic heterogeneity. In the study reported here selected individuals from five extended South African FSHD families were genotyped with nine short tandem repeat polymorphic (STRP) markers: D4S1652, D4S2930, D4S2390, D4S2921, D4S426, D4S2299, D4S2688, D4S1523, and D4S2283. These nine STRP markers span a region of ca. 4.67 cM. The South African FSHD families presented in this study consists of 3 - 9 generations, from which individuals of two to three generations were genotyped. Integrated haplotypes were constructed for 74 individuals. Identical FSHD-associated haplotypes were observed in two families (F10 and F30). However, the FSHD-associated haplotype of two other families (F20 and F60) differed from those observed in F10 and F30. Results from this pilot study therefore indicate the presence of at least two distinct FSHD-associated haplotypes in the South African FSHD population. The definitive criteria for the molecular diagnosis of FSHD are based on Southern Blot analysis. Southern blot analysis has been performed for core individuals from the extended families enabling molecular confirmation of the, to date, clinical diagnosis of FSHD in the South African population.

Genomic screen for adult onset glaucoma susceptibility genes: Fine mapping of candidate regions. *J.L. Wiggs¹, J.L. Haines², L.R. Bailey², J. August¹, E.A. DelBono¹, B. Broome⁴, K. LaRocque³, F. Lennon Graham³, M. Hauser³, M. Pericak-Vance³, R.R. Allingham⁴.* 1) Dept Ophthalmology, New England Medical Ctr, Boston, MA; 2) Program in Human Genetics, Vanderbilt School of Medicine, Nashville, TN; 3) Center for Human Genetics, Duke University School of Medicine, Durham, NC; 4) Dept. of Ophthalmology, Duke University School of Medicine, Durham, NC.

Previous studies have suggested that multiple genetic defects and possibly environmental factors may influence adult onset glaucoma susceptibility. To identify the chromosomal locations of adult glaucoma susceptibility genes, we have completed an initial genome screen using two independent pedigree sets (182 total affected sibpairs). Our initial studies identified 16 chromosomal regions that demonstrated interesting results (lod score >1.0 and/or a p-value <0.05). Further studies including the second independent pedigree set identified regions on chromosomes 2, 6, 9, 11, 14, 17 and 19 that continued to produce model-dependent lod scores and/or an MLS > 1.0 , while five regions (2, 14, 17p, 17q and 19) produced an MLS > 2.0 (Wiggs et al., Hum Molec Genet 9:1109-1117). The purpose of the current study is to further refine these chromosomal regions with a third set of thirty affected sibling pairs and additional markers spanning the candidate chromosomal region at 2-5 cM intervals. Linkage analysis was performed using both model-dependent (lod score) and model-independent affected relative pair and sibpair methods. Because of the relatively small number of pedigrees, the data from the third pedigree set was analyzed together with the data from the first two pedigree sets. Analysis of the data indicates that markers located in the candidate regions on chromosomes 14 and 19 continue to show positive results. SNPs in three candidate genes located in the chromosome 14 region have been identified to be used for linkage disequilibrium testing. Further analyses of the other candidate chromosomal regions are currently underway.

The effects of misspecification of marker allele frequencies in affected sibpair linkage analysis. *B.A. Thiel, H. Tiwari, D. Fallin, N.J. Schork.* Epidemiology/Biostatistics, Case Western Reserve Univ, Cleveland, OH.

Affected sibpair (ASP) linkage analyses are often used to identify the rough genomic location of disease predisposing loci. Although of great scientific value and sampling convenience, ASP designs can be problematic when parental genotype information is not available. This is due to the fact that in order to compute relevant allele sharing probabilities in the absence of parental genotype information, one must rely on accurate marker allele frequencies. In this study we investigate the effect of marker allele frequency misspecification on ASP test statistics using simple analytic derivations. We consider the case of a single biallelic marker locus for two situations. The first situation involves general inaccuracy of allele frequencies that might arise, for instance, when estimating frequencies from family data without regard to dependencies within the families. We have compared allele frequency estimation methods and show the degree to which these different estimates can effect the sharing probabilities and test statistics in a linkage analysis. The second situation involves allele frequency misspecification of the type that would arise in samples of sibpairs manifesting cryptic stratification in which individuals are chosen from populations with different underlying allele frequencies. We show that the false positive rate of ASP tests that make use of misspecified marker allele frequencies can be substantial in certain situations. We discuss the implications of our results and also comment on the degree to which multipoint strategies and the use of multiallelic marker loci can overcome these problems.

Evaluation of Linkage to the GLC1E Locus in 46 Glaucoma Families and Mutation Screening of GATA3, IL2RA, IL15RA and NAPOR in a Linked Family. *T. Rezaie*^{1,2}, *A. Child*², *G. Brice*², *T. Desai*¹, *G. Walls*², *R. Pitts Crick*³, *M. Sarfarazi*¹. 1) Molecular Ophthalmic Genetics Laboratory, Department of Surgery, University of Connecticut Health Center, Farmington, CT; 2) Department of Cardiological Sciences, St. George's Hospital Medical School; 3) International Glaucoma Association, King's College Hospital, London, UK.

We have previously reported mapping of a locus (GLC1E) for the low-tension form of Primary Open Angle Glaucoma (POAG) to the 10p15-p14 region. Recently, we screened 46 POAG families with mixed low and high intraocular pressures (IOP). Thirty-two families are unlinked. One typical low-tension caucasian and 2 black families (1 Gambian, 1 Jamaican) with mixed IOPs segregate for the entire region. Eleven other families are presumably linked and require further saturation mapping. Therefore, the GLC1E locus may potentially be involved in mixed low and high tension Glaucoma families in both white and black populations. Four genes from the GLC1E region were screened in one large linked family. For GATA3, no causative mutation was present in the coding sequences but a single SNP (AAC->AAT) was detected at amino acid #294. For IL2RA and IL15RA no sequence changes were observed. Screening of Neuroblastoma APOptosis-Related RNA-binding (NAPOR) protein identified a new isoform (NAPOR-4) that consists of a 6 amino acid (VAQMLS) deletion at positions 335-340 (like NAPOR-3) and a 4 amino acid (TINS) insertion after glycine at position 341. Expression of NAPOR-4 was observed in brain, retina, fetus, fibroblasts and lymphocytes. BAC sequencing identified no introns in this region thus suggesting that this isoform is not due to splicing error. Furthermore, as both homozygote and heterozygote NAPOR-4 isoforms were observed in our family and in a control population, this was considered to be a polymorphism. This provided further evidence for exclusion of NAPOR as a potential disease-causing gene for this phenotype. Mutation screening of other genes is currently in progress. Supported By National Eye Institute (EY-09947); UCHC-GCRC (M01RR-06192); International Glaucoma Association; Royal National Institute for the Blind and Bluff Field Charitable Trust, UK.

A new locus for dominant progressive hearing loss DFNA37 mapped to chromosome 1p21. *Z. Talebizadeh¹, J.B. Kenyon², J.W. Askew¹, S.D. Smith².* 1) Genetics, Boys Town Natl Res Hosp, Omaha, NE; 2) University of Nebraska Medical Center, Omaha, NE.

In this study we have shown linkage of dominant progressive hearing loss in a 4-generation American family (family 513) to chromosome 1p21. Linkage analysis with markers from ABI Prism panels produced a lod score of 8.29 for marker D1S495 at $q=0.0$. Hearing loss appears to be completely penetrant, and affected individuals experience an early-onset high frequency hearing loss which progressed with age to include middle and lower frequencies. The craniofacial features of affected family members are entirely normal, and there is no history of ocular abnormalities nor cleft palate in the family. Affected individuals do appear clinically to have some widening of the epiphyses of the long bones, however X-rays from the proband ruled out any abnormalities in his knees. The COL11A1 gene is one of the genes in the linked region. Both linkage analysis and involvement of the COL11A1 gene in Marshall and Stickler syndromes suggested this gene as a strong candidate for mutation analysis in family 513. The entire coding region of COL11A1 was screened and this collagen gene was strongly excluded for this family. A shaw-related potassium channel gene, KCNC4, was also considered as a candidate gene. No mutation was observed in the pore region of KCNC4, in association with hearing loss in family 513. The linked region is being further searched for another candidate gene. To date, there has not been any report of linkage of non-syndromic hearing loss to chromosome 1p21. This new locus is designated as DFNA37. Since the only gene involved in hearing loss in this chromosomal band is COL11A1, which was excluded in family 513, identification of the deafness gene in this family will add a new gene involved in auditory function.

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A New Test for Linkage in Affected Sib Pairs. *S.S. Shete, C.I. Amos.* Epidemiology, Box 189, UT MD Anderson Cancer Center, Houston, TX.

In affected sib pair linkage methods the mean proportion of marker alleles shared identical by descent (ibd) among affected sib pairs has been shown to be a powerful test for detecting linkage compared with other tests for most situations. However it is not always possible to estimate ibd sharing between sibs unambiguously, even when the parental genotypes are known. Here we define a measure of informativity for scoring ibd sharing based on the parental mating and sib pair genotypes. This measure reflects the precision with which one can obtain the ibd sharing information between sibs based on family data. Then we define a new weighted mean proportion of alleles shared ibd by sib pairs test statistics. Here we assign precision for estimating the ibd sharing as weights to the proportion of alleles shared ibd by affected sib pairs. Simulation results and its implications are discussed.

Linkage and candidate gene analysis of two pedigrees with Charcot-Marie-Tooth Neuropathy Type1C. V.A.

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Charcot-Marie-Tooth neuropathy type 1C (CMT1C) designates a rare form of autosomal dominant demyelinating CMT that has not been mapped genetically. Affected individuals in two families (K1550 and K1551) with CMT1C manifest characteristic CMT symptoms including high-arched feet, distal muscle weakness and atrophy, depressed deep tendon reflexes, sensory impairment, and slow nerve conduction velocities. The three genes peripheral myelin protein-22 (PMP-22), myelin protein zero (MPZ), and early growth response 2 gene (EGR2) known to cause CMT1 have been excluded as candidates for causing CMT1C. We are conducting a full genome scan to determine the chromosomal location harboring the CMT1C gene(s).

Clinical characterization and gene mapping of a family with X-linked mental retardation, facial dysmorphism, congenital hip dislocation and skewed pattern of X-inactivation. *N.J. Carpenter^{1,2}, H. Givens¹, L. Randell¹, R.*

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Gene mapping studies were performed on a four-generation family with X-linked mental retardation (XLMR). The syndrome is characterized by moderate mental retardation, congenital hip dislocation, microcephaly, dysmorphic facial features including wide nasal bridge and hypertelorism, low set ears and short neck. Short sternum, hypotonia and abnormal hair whorls with abnormal hair patterning are also observed in affected individuals. Four obligate carrier females are physically normal and are not retarded. The haplotypes of twelve family members were determined using lymphocyte DNA and PCR amplification of 22 dinucleotide repeat polymorphic loci located throughout the length of the X chromosome. Two-point and multi-point linkage analyses were performed using FASTLINK (version 3.0P). Maximum lod scores were observed at $q=0.0$ for DXS1220 (lod score = 2.14), DXS424 (lod score = 2.23) and DXS1001 (lod score = 2.087). Recombinations were detected between DXS1220 and DXS424 and between DXS1001 and DXS1212 defining the proximal and distal boundaries of a candidate gene region in Xq23-Xq24. X-inactivation patterns were determined for five females by assessing differential methylation patterns of the CAG repeat in the first exon of the androgen receptor gene. X-inactivation was completely skewed in four obligated carriers and random in one female who does not appear to be a carrier by haplotype analysis. These results imply that this XLMR gene is expressed in lymphocytes and that a selection process occurs during embryogenesis that favors the lymphocytes expressing the normal gene product.

A Genomic Screen For Dementia In An Extended Amish Family. *J.L. Hall¹, A. Ashley-Koch², P.C. Gaskell², C.E. Jackson³, L.R. Bailey¹, W.K. Scott², J.L. Haines¹, M.A. Pericak-Vance².* 1) Vanderbilt University Med Ctr, Nashville, TN; 2) Duke University Medical Center, Durham, NC; 3) Henry Ford Hospital, Detroit, MI.

Late-onset Alzheimer disease (LOAD) is phenotypically and genetically complex. While many genes have been proposed as modulating the risk of LOAD, only the effect of APOE has been confirmed while another gene that lies in the centromeric region of chromosome 12 remains unidentified. Large families with LOAD are rare, but can be extremely useful in localizing at least one gene. We identified such a family in the Indiana Amish population. Although the dementia in the Amish is clinically indistinguishable from LOAD, social beliefs prevent autopsy confirmation of an LOAD diagnosis. Previous analyses of this Amish population demonstrated a significantly decreased frequency of the APOE-4 allele (Pericak-Vance, 1996) but significant familial clustering of dementia suggesting a different genetic etiology. Linkage to the known AD loci (APOE, APP, PS1 and PS2) was excluded. Therefore, we embarked on a genomic screen to identify novel dementia susceptibility loci in this family. We screened 24 individuals (7 affected) for 296 markers. Due to extreme pedigree complexity, we were restricted to two-point linkage analyses. Both model-dependent "affecteds-only" analysis (dominant and recessive) and model-independent affected relative pair analyses were employed. Critical values ($\text{lod} > 1.0$ and/or $p < 0.05$) were obtained on 22 markers over 11 chromosomes. Three markers yielded critical values for both model-dependent and model-independent analyses: D4S2379 ($\text{lod} = 1.22$, $p = 0.04$), D8S1128 ($\text{lod} = 1.51$, $p = 0.01$), and D10S202 ($\text{lod} = 1.30$, $p = 0.03$). The highest lod score was obtained at marker D22S1685 ($\text{lod} = 1.62$) and the smallest p-value was obtained at marker D7S517 ($p = 0.003$). Of particular interest is the lod score for D12S1042 ($\text{lod} = 1.25$) and for D4S1629 ($\text{lod} = 1.25$), the same markers yielding substantial two-point lod scores in a previous AD genomic screen (Pericak-Vance, 1997). These regions have been prioritized for examination in additional Amish families collected in both Indiana and Ohio.

Fine Mapping of Asthma Genes. *M. Wjst, T. Immervoll, S. Loesgen, H. Bickeboeller, for. the German Asthma Genetics Group.* Inst fuer Epidemiologie, GSF Forsch Umwelt & Gesundh, Neuherberg Munich, Germany.

Four genomwide searches have been reported until now for asthma and related phenotypes [1]. In an own study of affected sib pairs of German and Swedish families we have found positive linkage results for asthma on chromosome 2, 6, 9 and 12 [2]. We report now fine mapping of these regions plus candidate regions on chromosome 5, 7 and 11 previously not linked in this study. The study included families with at least 2 children between age 6 and 18 with asthma recruited from several university hospitals. All participants completed a detailed interview, had a complete bronchial challenge with methacholine and recorded for 10 day their peakflow. Skin prick tests included 11 allergens which were also tested in RAST assays together with total IgE. Microsatellite typing was done with a panel of additional 52 markers spaced in approximately 2 cM distance. Our previous linkage regions could be confirmed while additional regions were also negative in the denser map. Linkages for asthma on chromosome 2, 6, 9 and 12 all increased. The most prominent increase of an asthma associated trait was seen for a quanatitative analysis of peripheral eosinophil count at marker D6S16411 with $p = 5.3 \times 10^{-5}$. Initial association with candidate genes in this region were negative.

[1] Asthma Gene Database <http://cooke.gsf.de>

[2] Wjst et al. Genomics 1999; 58: 1-8 [BR>Supported by GSF #73922.

The SOD1 gene is not involved in sporadic Amyotrophic Lateral Sclerosis. *T. Siddique*¹, *S. Schmidt*², *B. Hosler*³, *W.-Y. Hung*³, *W. Chen*¹, *A. Thierry*¹, *N. Siddique*¹, *Y. Bradford*⁴, *J.L. Haines*⁴, *M.A. Pericak-Vance*², *R.H. Brown*³. 1) Northwestern Univ Medical Sch, Chicago, IL; 2) Duke Univ Med Ctr, Durham, NC; 3) Massachusetts Gen Hosp, Boston, MA; 4) Vanderbilt Univ Med Ctr, Nashville, TN.

Amyotrophic lateral sclerosis (ALS) is an age-dependent neurodegenerative disorder of motor neurons with both sporadic (~90%) and familial (~10%) forms. Onset is generally in mid-life with an average survival of 3-5 years. A subset of familial ALS is autosomal dominant, and approximately 20% of autosomal dominant ALS is due to mutations in the Cu/Zn superoxide dismutase (SOD1) gene (Siddique et al. 1991, Rosen et al., 1993, Deng et. al., 1993). This indicates that SOD1 plays a functional role in the etiology of ALS. While sporadic ALS patients do not have causative mutations in the SOD1 gene, polymorphisms within the gene might modulate its biological action and thus also modulate the risk of developing sporadic ALS. To investigate this possibility, we performed a familial association analysis of four polymorphic markers within the SOD1 gene on families of 148 sporadic ALS cases. Cases, parents, and/or unaffected siblings were genotyped for the four markers, producing both triad- and discordant sibship data useful for linkage disequilibrium analyses. The pedigree disequilibrium test (PDT; Martin et al., 2000) combines these two family types into a single valid test. To test for association only with respect to maternally or paternally transmitted marker alleles, the classical TDT for triad data was employed as well. Transmission of multimarker haplotypes was tested with the Transmit program (Clayton 1999). Informative samples sizes ranged from 104 to 125 triads and 1 to 22 discordant sibling pairs per marker. The global PDT results for all markers were non-significant (ranging from 0.76-0.39). Tests of non-random maternal or paternal transmission were also non-significant, as were the tests of transmission of two-marker haplotypes. We conclude that variation in the SOD1 gene is unlikely to play a substantial role in the etiology of sporadic ALS.

A statistical method for identification of a functional polymorphism in a gene. *L. Sun¹, N. Cox², M.S. McPeck¹*. 1) Department of Statistics, University of Chicago, Chicago, IL; 2) Department of Human Genetics, University of Chicago, Chicago, IL.

Consider a locus affecting a qualitative trait that has been localized to a rather narrow region by linkage analysis or linkage disequilibrium mapping. At this point, one may be able to identify one or several genes within the region. Even if only a single gene lies in the region, it may contain a large number of polymorphic sites. We consider the problem of determining which SNP or combination of SNPs influence the trait. We focus on the sib-pair study design. Our approach is based on the observation that if a particular site is the only site in the region that influences the trait, then conditional on the genotype at that site for each sib pair, there should be no excess sharing by sibs in the region. We consider a model that is a variation on the usual allele-sharing methods, and we derive a confidence region for the site influencing the trait, under the model that assumes there is only one site in the region influencing the trait. We also consider the problem of multiple sites influencing the trait. We extend the method to larger sibships and apply it to the NIDDM1 data set of Horikawa et al. We consider 170 families typed at 26 SNPs that span approximately 100 kb but are mostly concentrated in a 65 kb region containing two loci. The results suggest that among the SNPs we consider, no single SNP can explain the linkage results.

Multipoint fine-scale linkage disequilibrium mapping by the Decay of Haplotype Sharing using marker genotypes rather than haplotype data. *A. Strahs¹, N. Cox², M.S. McPeck¹*. 1) Department of Statistics, University of Chicago, Chicago, IL; 2) Department of Human Genetics, University of Chicago, Chicago, IL.

We discuss the application of the Decay of Haplotype Sharing (DHS) method for assessment of linkage disequilibrium to fine-scale genetic mapping. Emphasis is on the case in which marker genotypes from affected individuals, in lieu of haplotypes, as well as marker allele frequencies or haplotypes or genotypes from appropriately selected controls, are available for study. This methodology may be particularly important for mapping studies for diseases with a late-onset, or other cases in which it is problematic to obtain haplotype information. Rather than estimate affecteds' haplotypes and apply haplotype-specific multipoint methods, we incorporate uncertainty about phase into the hidden Markov model, in which the embedded Markov chain models the recombination and mutation processes and the observations are the genotypes at the marker loci. The estimate of the location of the gene is obtained by maximum likelihood, or maximum quasi-likelihood. We apply this method to the NIDDM1 dataset of Horikawa et al. For this study, we consider 70+ SNPs and microsatellite markers across 1.6 Mb for 108 affecteds and 109 control individuals. The DHS methods, for haplotype and genotype data, are currently being implemented in publicly available free software.

Genetics of Fahr's disease: refining the *IBGCI* locus and candidate gene analysis. *M.J. Sobrido*¹, *M. Baquero*², *D.H. Geschwind*¹. 1) Department of Neurology, UCLA, Los Angeles, CA; 2) Department of Neurology, Hospital Universitario La Fe, Valencia, Spain.

The eponym Fahr's disease is commonly used in reference to the idiopathic diffuse calcification of the basal ganglia (IBGC), a neurodegenerative syndrome sometimes presenting in families. We have previously reported that at least one gene responsible for this condition resides on the *IBGCI* locus on chromosome 14q (Geschwind et al., 1999). We hypothesized that a triplet repeat expansion may be the causal mutation, since clinical anticipation is present in the *IBGCI*-linked pedigree and appears to be present in other IBGC families as well. In the context of the Human Genome Sequencing Project, about 70% of chromosome 14q sequence has been released in form of either draft or complete sequence.

PURPOSE: To refine the *IBGCI* locus, evaluate potential triplet-repeat expansions and examine candidate genes in the region.

METHODS: We analyzed additional families with calcification of the basal ganglia for linkage to markers in the *IBGCI* region. Computational genomic tools, including the program GeneQuest® (DNASTAR) and the annotation workbench Genotator (Harris NL, 1997), were used to examine the available sequence in the critical region.

RESULTS: One additional pedigree of Spanish origin showed potential linkage to the *IBGCI* locus. The most critical region, spanning 11.7 cM between markers D14S75 and D14S989, is represented by 9 sequenced BAC contigs and 170 overlapping BACs. Possible candidate genes include GCH1, PSMA6, SSTR1, KTN1, AKAP100 and MPPB. We have identified 14 (CAG)_n sequences longer than four repeats that we are currently screening for mutations in affected family members.

Flexible multiplexed genotyping assay format that combines a novel labeling strategy with microcapillary electrophoresis. *M.T. Cronin¹, S. Williams¹, Y. Wang¹, M. Pho¹, J. Wei¹, S. Leon¹, T. Matray¹, S. Singh¹, K. Livak², E.S. Mansfield¹.* 1) ACLARA Biosciences, Inc, Mountain View, CA; 2) PE BioSystems, Foster City, CA.

Current genetic analysis needs range from genome-wide SNP scans for large association studies to specific genotyping panels used for phenotype prediction. No technology presently available efficiently addresses all of the performance and throughput needs associated with this range of applications. In addition, no current technology has the flexibility to respond effectively to today's evolving analytical landscape where new polymorphism targets continuously become available. By combining a proprietary new probe labeling strategy with existing, robust genotyping biochemistries and ACLARA's LabCard™ micro-capillary electrophoresis separations systems, we have designed a flexible, multiplexed assay format for analyzing nucleic acids in a broad spectrum of genomics applications. This assay configuration is uniquely characterized by its modular composition. Sets of electrophoretic mobility tags (e-Tags™) are used as labels to "code" multiplexed probe sets for genotyping or expression reporter assays. These tags are released during 5' exonuclease cleavage assays and are subsequently separated by microcapillary electrophoresis in channels in plastic LabCard devices or on standard capillary electrophoresis systems. The electrophoretic pattern is "decoded" to yield genotypes or gene expression patterns. This assay strategy is being applied to develop multiplexed genotyping assay capability for pharmacogenetics applications. Initially, 20 e-Tags will be bundled to provide 10-plex genotyping capability during each capillary channel separation. These assays are being optimized to run in parallel on analytical capillary instruments and on ACLARA LabCard devices currently in development. The modular assay design allows a single genotyping multiplex to be applied to a large number of samples or, alternatively, parallel multiplex reactions can be done to assay a large number of polymorphisms in a single sample. Multiplexed e-Tag genotyping data for the pharmacogenetics targets CYP2D6 and ApoE will be presented.

Discovery and scoring of single nucleotide polymorphisms within a bipolar disorder candidate region on chromosome 18q21.3-22. *Y-S. Chen, C. Yarnes, F.J. McMahon.* University of Chicago, Chicago, IL.

Considerable discussion has occurred in recent years concerning the use of single nucleotide polymorphisms (SNPs) and association to find loci contributing to common, complex genetic disorders. Bipolar affective disorder (BPAD) is one such disorder, characterized by recurrent cyclic episodes of mania and depression. A susceptibility gene for BPAD may reside on chromosome 18q21.3-22. Based on multipoint linkage results using a dense set of microsatellite markers, we have selected a 14 cM region as a starting point for linkage disequilibrium mapping of this gene. We have selected 24 sequenced BAC clones and 42 sequence tagged sites (STSs) for SNP screening by DNA re-sequencing. PCR primers were designed to amplify ~1 kb of sequence every ~10 kb across each BAC. The BAC and STS sequences were PCR-amplified from a panel of 12-16 unrelated subjects (3-8 probands from 18q-linked families and 6-9 CEPH controls), the PCR product was purified, concentrated, and the forward strand was sequenced on an ABI 377 or 3700 sequencer. Pherograms were then compared visually for candidate SNPs, based on the presence of 2 peaks at a single nucleotide position. Forty SNPs has been discovered through 38 kb of unique DNA screened (1 SNP per 0.95 kb). In addition, about 120 SNPs have been found accross the region through mining of public databases. We are in the process of genotyping a selection of these SNPs within the region. Maps, genotyping, and association results will be presented.

Characterization of two unusual polymorphisms identified by sequence comparison. *Y. Watanabe*¹, *J.C. Murray*^{1,2}, *B.C. Bjork*², *C.P. Bird*³, *P.-W. Chiang*⁴, *S.G. Gregory*³, *D.M. Kurnit*^{4,5}, *B.C. Schutte*^{1,2}. 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Program in Genetics, Univ Iowa, Iowa City, IA; 3) Sanger Center, Hinxton, Cambridgeshire. UK; 4) Dept Pediatrics, Univ Michigan, Ann Arbor, MI; 5) Dept Human Genetics, Univ Michigan, Ann Arbor, MI.

We reported an 8kb deletion/insertion (D/I) polymorphism in the Van der Woude syndrome critical region at 1q32-q31 at the previous meeting. Embedded in this 7921 bp region was a TTCC repeat. Genotype analysis showed that both the internal short tandem repeat and the 8 kb deletion/insertion mutation were true polymorphisms. This is a novel example of intraallelic variation. In this report further genetic and DNA sequence analysis indicated that the ancestral state of the 8 kb D/I polymorphism was the insertion allele and that the original deletion mutation probably occurred only once. The finding that the D and I alleles are in linkage disequilibrium with alleles from nearby markers supports that the 8kb D/I polymorphism is an old mutation. A second polymorphism was discovered in 328 bp of sequence completely identical at loci on chromosome 1 and 5. In the chromosome 5 sequence an A/G single nucleotide polymorphism was identified that has not been found in any of 126 chromosomes 1 as assayed by ARMS using chromosome 1 and chromosome 5 specific sequence outside the homology region. We propose to call these new classes of polymorphisms "Matroshka" and "ectopic" polymorphisms. A "Matroshka" polymorphism is a form of intraallelic variation with one polymorphism nested within a second polymorphism and is named for the Russian doll that has additional dolls nested inside. An "ectopic" polymorphism is a polymorphism that is located in sequence that is duplicated with the exception of the polymorphic site at an independent locus. These two new classes of polymorphisms are important because they may confound genetic and sequence analyses and may also contribute to variation in disease phenotypes.

Sniper: An automated system for high throughput SNP scoring. *E. Roschmann, F. Ghouze, R. Howe, A. Bamford, E. Shipstone, B. Hughes, G. Scozzafava, S. Strong.* Amersham Pharmacia Biotech, Amersham, Buckinghamshire, UK.

It has been proposed that association studies using common sequence variants or single nucleotide polymorphisms (SNPs) may provide a powerful approach to dissect the genetic inheritance of common complex traits. This requires a cost-effective, high throughput technology for scoring SNPs enabling the determination and analysis of sequence differences between individuals and populations for a large number of SNPs. An enabling technology for these applications is Sniper™, a high throughput SNP scoring system which uses Rolling Circle Amplification (RCA) to score SNPs from nanogramme amounts of genomic DNA in a homogenous microtitre plate format. The whole process will be automated to the required high throughput, with a target of 500,000 genotypes per day. The integrated Sniper system, employing robotics and laboratory workflow systems, aims to address the throughput, cost, accuracy and DNA consumption constraints currently limiting the SNP-based identification of sequence variants associated with disease susceptibility and drug response genes.

Reducing size bin variation using allelic ladders. *M.W. Perlin^{1,2,3}, M.A. Clarke¹, M. Breen¹, N.A. Zezza², S.Y. Hill².*
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DNA fragment analysis measures molecular size in order to estimate the lengths of different DNA polymers. Such analyses are useful in many genetic applications, including STR, SNP, SSCP, AFLP, and differential display studies. The fragment sizing is done by measuring the DNA migration relative to internal size standards, and then reporting the fractional interpolated size. Unfortunately, the relative migration between the assayed DNA and the size standards is highly variable between size separation runs, particularly when different DNA sequencer instruments or platforms are used. This can lead to size bin variation that exceeds $\pm 0.5\text{bp}$, causing uncertainty in the DNA length determination.

The DNA forensics community has long addressed this inter-run sizing variability by using intra-run allelic reference ladders for 5-15 standard tetranucleotide repeat markers. Their approach becomes more challenging in genetic studies, where 100-1000 STRs may be used, including di- and tri-nucleotide repeats.

We have adapted the allelic ladder (AL) approach for use in genetic STR analysis. For every marker, we pool PCR products from a set of samples to define a reference AL. We include this pooled AL in a lane on all succeeding electrophoresis runs, thereby creating a multi-allele common size bin reference. We use our TrueAllele genotyping analysis software to automatically compare each sample's DNA peaks with the pooled AL reference peaks. The result is a reduction in size bin variation that allows us to more confidently estimate DNA fragment length across many different electrophoretic sizing runs.

Analysis of SNPs using complete hydatidiform moles by microchip arrays. *U. Surti¹, J. Fan³, P. Taillon-Miller², L. Hsie³, T. Ryder³, D. Mutch², P. Kwok².* 1) Pathol/Gen, Magee Women's Hosp, Univ Pittsburgh, Pittsburgh, PA; 2) Washington University School of Medicine, St. Louis, MO; 3) Affymetrix, Santa Clara, CA.

Complete Hydatidiform mole represents a unique experiment of nature that is thought to result from the fertilization of an empty ovum by a haploid 23,X sperm that duplicates its genome to give rise to 46,XX genotype of androgenetic origin. Genomic DNA samples from 5 CHMs and the corresponding decidua representing the maternal samples were genotyped with 1494 markers on the HuSNP2K microarray. Each sample gave definite unambiguous genotypes for >1200 SNP markers. The maternal samples were found to be heterozygous at more than 25% of the markers where as the 5 CHM samples were heterozygous at less than 1% of the SNP markers. The level of heterozygosity in the CHM samples was below the expected experimental error rate in the HuSNP2K microarray genotyping method. The 5 sets of genotypes of the CHMs represent the haplotypes of their genomes. Several regions of the haplotypes were shared by two samples over a 50cM distance. In our study of >1200 SNP markers, we confirm our expectation of the homozygous nature of CHMs. Previous studies were based on a limited number of markers. These results offer an opportunity to gain insight into the regions of linkage disequilibrium in the genome. Our results also demonstrate the utility of CHMs in validating the SNPs used for the construction of SNP microchip arrays.

An investigation of HLA-DR2 and clinical phenotypes in familial multiple sclerosis (MS). *L.F. Barcellos¹, J.R. Oksenberg¹, A.J. Green¹, P. Bucher¹, R.R. Lincoln¹, M.E. Garcia², J.B. Rimmeler³, J.L. Haines², M.A. Pericak-Vance³, S.L. Hauser¹.* 1) Dept of Neurology, University of CA, San Francisco; 2) Program in Human Genetics, Vanderbilt University; 3) Center for Human Genetics, Duke University.

MS is a clinically heterogeneous demyelinating disease of the CNS characterized by axonal loss and progressive neurological dysfunction. A complex genetic susceptibility plays a clear role in disease etiology. The HLA-DR2 haplotype within the MHC on ch.6p21 has consistently demonstrated both linkage and association with MS; however, the role of DR2 in determining clinical features of MS is unclear. Rigorous criteria were employed to collect 174 Caucasian MS families (464 affecteds), and a detailed evaluation of patient records was undertaken to identify clinical phenotypes. Linear and logistic regression analyses were used to evaluate DR2 influences on clinical outcomes. Several MS phenotypes were examined including disease subtypes and age of onset. A total of 73.7% of patients were classified as "disseminated MS" (57.4 % of families) and 26.3% of patients presented with an "opticospinal" form of MS (10.8 % of families). A group of benign MS patients (EDSS <3 after 10 yrs) was also identified (11.0% of patients, 3.4% of families). In addition, patients and families were also categorized by first symptoms (site of onset); 49.4% of patients (24.3% of families) presented with optic nerve or spinal cord involvement. Linkage and association to HLA-DR (MLS=2.9, q=0.15; p<0.001 respectively) was observed in the families. While male patients had a later mean age of onset than females, differences in onset age or gender were not observed in any phenotypes. Further, no associations between DR2 and any clinical phenotypes examined in patients, or linkage in families stratified by phenotypes were observed. While DR2 plays an important role in MS susceptibility, our results indicate it does not influence the specific clinical outcomes examined here. In addition, the more clinically homogeneous subgroups of MS families identified here will be invaluable to our candidate gene and genome screening efforts that are currently underway.

Uteroglobin Gene - Polymorphism and Genetic Susceptibility to Asthma. *M.S. Choi, Z. Zhang, A.B. Mukherjee.*
Heritable Disorders Branch, NICHD/NIH, Bethesda, MD.

Uteroglobin(UG) is a multifunctional secreted protein with antiinflammatory/immunomodulatory properties. Asthma is an inflammatory disease of the airways, in which UG is constitutively expressed. During the past five years, several laboratories have reported the existence of potential candidate asthma susceptibility loci on human chromosomes. Most studies have focused on identifying candidate loci on chromosome 5q31-33 and 11q13 either by linkage analysis or by association studies. A putative asthma susceptibility gene locus has been reported on human chromosome 11q13, the same region in which the human UG gene is localized by FISH. In order to identify genetic factors contributing to asthma susceptibility, we conducted a genetic association study of the UG gene in 22 families with asthmatic children. We found that in the noncoding region of exon 1, there is a polymorphism (+38 A->G) that could be detected by PCR/Sau96I restriction endonuclease digestion. Our results show that there is a significant increase in the frequency of the +38 A->G allele in asthma patients compared with non-asthmatic controls. In addition to this polymorphism we also identified a novel (GTTT)_m STR polymorphism adjacent to the (ATTT)_n STR region at around -3100 bp from transcription start point of the UG gene. Genotyping of these patients is carried out with these STR polymorphic sites by PCR-FLP and sequencing. There is a significant difference in the allele frequencies of (GTTT)_m polymorphism in asthma patients compared with non-asthmatic controls. Taken together, our results raise the possibility that UG gene polymorphisms [A38G and (GTTT)_m] may predispose to airway inflammatory diseases, such as asthma. Investigation with a larger asthma patient population is essential to confirm these results.

Myelin Oligodendrocyte Glycoprotein polymorphism frequency in Multiple Sclerosis patients. *M. Gomez Lira¹, A. Salviati², G. Moretto³, B. Bonetti², M.D. Benedetti², P.F. Pignatti¹*. 1) Dpt Mother-Child, Biol-Genetics, and; 2) Dpt Neurological Sciences, Univ. Verona; 3) Neurology Unit, Hospital Belluno, Italy.

Multiple sclerosis (MS) is a demyelinating disorder with an autoimmune etiology. It is thought to be a multifactorial disease with a complex mode of inheritance. Genomewide screens of MS families have reported several provisional sites, but only two chromosomal regions, 6p21 and 5p, were positive in more than one data set. Beside the HLA complex, the 6p21 region contains the gene coding for the myelin oligodendrocyte glycoprotein (MOG). MOG is located in the outer layer of the myelin sheet. MOG is a member of the immunoglobulin super family, and may act as an autoantigen in MS. We have recently identified three new polymorphisms in the MOG gene: G15A, located in the signal peptide in exon 1, Val142Leu in exon 3, and 571+68A/G in intron 4. We have genotyped 50 MS patients and 50 unaffected control individuals from the Italian population for these three polymorphisms. While polymorphism G15A presents no association with MS, polymorphism Val142Leu and 571+68 A/G gave a significant allele frequency difference between MS (7/100) and normal individuals (18/100) (Fisher exact Test: $p = 0.03$). These preliminary data offer new indication that MOG may be involved in the pathogenesis of MS. We are expanding this research to a larger number of individuals for confirmation.

Transmission Disequilibrium Tests in Extended Pedigrees. *M. Karnoub, D. Zaykin, D. Nielsen, M. Ehm.* GLAXO WELLCOME, INC., RESEARCH TRIANGLE PARK, NC.

Transmission Disequilibrium Tests (TDTs) are being used to identify markers in close proximity to disease genes. Provided that a marker is associated with the disease, the TDTs have been shown to have more power to detect linkage than conventional linkage tests. Additionally, since the TDTs use within family controls, the potential bias due to population stratification is avoided. In the past 8 years, the TDTs were extensively studied because of the above attractive features. The developments were made to allow researchers to make use of the different data structures they were obtaining. We had 156 Causasian families collected for the Genetics in Diabetes project of the ADA. Faced with our varied family structures and patterns of missing genotypes, we went for a more general TDT method, implemented in Transmit (Clayton, 1999): this method is likelihood based and uses all available genotypes to reconstruct the missing parental genotypes. However, even though the method allows for the use of multiple affected siblings (while testing for association in the presence of linkage), it did not offer a fix for multiple generation families, which we thought could similarly affect the results. We ran an extensive set of simulations to test whether the Type I error rate and the power of the method were affected by leaving in the analyses all the nuclear families obtained from our extended pedigrees. The other option would have been to apply some pre-specified criteria on either the number of affected offspring or the number of genotyped parents, and to keep only one nuclear family per extended pedigree, and thus again to loose data. We also compared the results from Transmit to another recently submitted method, PDT (Martin et al.) that makes use of all the data in a TDT/Sib-TDT type of combination, without reconstructing genotypes. We describe the setup and the different models we used in our simulations, and thus justify our decision to leave our families intact.

Allele frequencies of cytokine gene polymorphisms in Caucasians and African Americans. *A-M. Martin¹, J.D. Greshock¹, T.R. Rebbeck², B.L. Weber¹.* 1) Dept Medicine, Univ Pennsylvania, Philadelphia, PA; 2) Center for Clinical Epidemiology and Biostatistics, Univ Pennsylvania, Philadelphia, PA.

A major goal in human genetics is to understand the role of common genetic polymorphisms in susceptibility to common diseases, including those involved in hereditary cancers, such as breast cancer. Polymorphisms in the coding and non-coding regions of genes of immune surveillance pathways may be involved in modulating the host's immune response to the presence of a tumor. Toward this end, we have evaluated the frequency of polymorphisms in immune surveillance genes among Caucasians and African Americans. We estimated allele frequencies for common polymorphisms in a number of cytokine genes including the TNF α promoter, IL1b, IL-1R, and IL-6 promoter. All of these genes play a major role in the immune response and some have been implicated in the immune response to tumors. Some of the polymorphisms tested have been shown to alter the function of the gene and expression of the gene product. The allele frequencies were estimated using a panel of 30 Caucasians and 28 African Americans. Significant differences in allele frequencies were found between the two ethnic groups for the following polymorphisms: IL-6 G-174C (p=0.002), IL-1RN T+11100C (p=0.012) and IL-1RN T+8006C (p=0.0004). We are currently investigating the association of some of these polymorphisms with age of diagnosis of breast cancer among BRCA1 mutation carriers. This will not only provide new information about other genetic factors that may be associated with breast cancer, but may generate hypotheses for biologic studies evaluating the role of these genes and associated variants in tumor surveillance genes.

Linkage disequilibrium mapping of an asthma locus near the Interleukin 1 complex on chromosome 2. E.

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We have previously described linkage and association of asthma to a region of chromosome 2 near the interleukin-1 cluster. Analysis of public domain polymorphisms identified a disease- associated microsatellite and suggested a locus conferring a two-fold increase in asthma susceptibility. Interleukin 1 and IL 1-RA were excluded as candidates. In order to identify the risk-increasing polymorphisms in this region we have sequenced 400 kilobases of DNA and identified candidate expressed sequences. We have mapped 103 single-nucleotide polymorphisms (SNPs) to the region, and genotyped 47 of these in 3 panels of caucasian individuals (48,404 genotypes). Analysis of single SNPs confirms association to asthma ($p = 0.002$), and associated phenotypes (lige, $p = 0.002$, rast index, $p = 0.004$). Here we present a combined analysis of multiple SNPs and haplotypes to explore the impact of this locus on asthma susceptibility. This joint analysis reduces the multiple testing problem and increases power, and will define an interval containing the disease gene. *Note: E. Noguchi and A. Heinzmann contributed equally to this work..*

Human adolescent nephronophthisis: Gene locus synteny with polycystic kidney disease in *pcy* mice. K. Haeffner¹, S. Burth¹, C. Fernandez², B. Fargier², A. Villaquiran², H.G. Nothwang³, S. Schnittger⁴, H. Lehrach³, D. Woo⁵, M. Brandis¹, R. Sudbrak³, F. Hildebrandt¹, H. Omran¹. 1) University Childrens Hospital, Freiburg, Germany; 2) University Hospital Los Andes, Venezuela; 3) Max-Planck Institute for Molecular Genetics, Germany; 4) University Hospital Grosshadern, Munich, Germany; 5) University of California, Los Angeles, USA.

We recently identified in a large Venezuelan kindred a new type of nephronophthisis: Adolescent nephronophthisis (NPH3) is a late-onset recessive renal cystic disorder of the nephronophthisis/medullary cystic group of diseases causing end-stage renal disease at a median age of 19 years. Using a homozygosity mapping strategy we previously localized the gene (*NPHP3*) to chromosome 3q22 within a critical interval of 2.4 cM. We here cloned the *NPHP3* genetic region and physically localized seven genes, eight ESTs and seven microsatellites within the critical disease interval. By human-mouse synteny analysis based on expressed genes we clearly demonstrated synteny between the human *NPHP3* locus on chromosome 3q and the *pcy* locus on mouse chromosome 9, thus providing the first evidence of synteny between a human and a spontaneous murine renal cystic disease. By fluorescence in situ hybridization we refined the chromosomal assignment of *NPHP3* to chromosome 3q21-q22. Renal pathology in NPH3 was found to consist of tubular basement membranes changes, tubular atrophy and dilatation, and sclerosing tubulointerstitial nephropathy. This pathology clearly resembled findings observed in the recessive *pcy* mouse model of late-onset polycystic kidney disease. In analogy to *pcy*, renal cyst development at the cortico-medullary junction was found to be an early sign of the disease. Through cloning of the *NPHP3* critical region and mapping of expressed genes we established synteny between human NPH3 and murine *pcy*, thus generating the hypothesis that both diseases are caused by recessive mutations of homologous genes.

Multiple-Marker-Locus and Multiple Trait-Locus Linkage Disequilibrium Mapping of Quantitative Trait Loci with Epistasis. *J. Zhao*¹, *C. Amos*², *E. Boerwinkle*¹, *M. Xiong*¹. 1) Human Genetics Center, University of Texas-Houston, Houston, TX; 2) M D Anderson Cancer Center, University of Texas, Houston, TX.

Epistasis plays an important role in genetic architecture of a trait and resurface from time to time in the literature. However, most widely used statistic methods for quantitative trait locus (QTL) analysis have focused on identifying individual QTLs and their effects, and ignore the interaction between the trait loci. In this report, we present physiological and statistical genetic models for epistasis and develop computational methods for the estimation of physiological additive, dominance and epistasis values for the physiological model, and genetic additive, dominance effects and additive additive, additive dominance, dominance dominance effects for the statistical genetic model. We establish relationship between parameters for the physiological model and parameters for the statistical genetic model. We carry out the power comparison of the tests for the presence of epistasis based on physiological and statistical genetic model and demonstrate that the physiological model has higher power to detect epistasis than the statistical genetic model. We extend the physiological model and statistical genetic model for epistasis at the trait loci to the marker loci and demonstrate that the epistatic effects at the marker locus for the statistical genetic model almost vanish even for the markers with the mild genetic distance from the trait locus. We apply the developed physiological and statistical genetic models to mapping QTL and propose general multi-marker-locus and multiple-trait-locus physiological and statistical genetic models for QTL analysis. We investigate the power of single-locus and multi-locus physiological and statistical genetic models for mapping QTL. We use feature variable selection methods for identification of trait loci. Finally, we give examples to illustrate the application of the proposed models to QTL analysis.

Suggestive linkage for endometriosis found in genome-wide scan. *S.A. Treloar^{1,2}, M. Bahlo^{2,3}, K. Ewen⁴, D.T. O'Connor¹, D.L. Duffy^{1,2}, C.A. Wicking^{2,5}, B.J. Wainwright^{2,5}, G. Montgomery^{1,2}, N.G. Martin^{1,2}.* 1) Dept Epidemiology, Queensland Inst Medical Res, Brisbane, Australia; 2) The Cooperative Research Centre for Discovery of Genes for Common Human Diseases, Australia; 3) The Walter and Eliza Hall Institute for Medical Research, Melbourne; 4) Australian Genome Research Facility, Melbourne; 5) The Institute for Molecular Bioscience, The University of Queensland, Brisbane.

We aim to identify genes predisposing women to the complex disease endometriosis. Our strategy for linkage and association analysis involves recruitment of 1,000 affected sister pairs (ASPs) diagnosed with the disease, and 1200 additional families with case-two parent control triads. Targets are based on power calculations assuming that endometriosis is oligogenic. Recruitment is Australia-wide, with some participants from New Zealand. We use the revised American Fertility Society criteria to determine stage of disease (I minimal, II mild, III moderate, IV severe), allowing for stratified analysis. Recruitment stands at over 550 ASPs and 650 triads.

Results from a genome scan by the Australian Genome Research Facility, using the ABI Prism Linkage Mapping Set Version 2 (PE Applied Biosystems) with 400 markers at ~10 cM coverage, are being analyzed with Genhunter 2.0 software (Kruglyak et al., 1996). To date, marker data have been generated and analyzed for a total of 289 ASP pedigrees containing 374 sib pairs, plus other affected relatives. Suggestive linkage has been identified for one locus, and possible linkage (Lander and Kruglyak, 1995) for 5 other loci. Our highest maximum-likelihood IBD estimate (MLS score), using combined data from five rounds of genotyping, and including all stages of disease, is 2.6. The non-parametric linkage analysis (NPL) score for this peak is 3.03.

Overlapping length and substitution polymorphisms in the promotor region of human tissue kallikrein gene (KLK1), and allele association analysis in hypertensive end-stage renal disease. *H. Yu¹, Q. Song², B.I. Freedman¹, J. Chao², L. Chao², D.W. Bowden¹.* 1) Biochem, Wake Forest Univ Sch Medicine, Winston-Salem, NC; 2) Biochem and Mol Biol, Med Univ South Carolina, Charleston, SC.

Kallikreins have long been studied in human essential hypertension and associated complications. We have been examining two kallikrein genes, KLK1 and KLKB1, in the African American patients with end-stage renal disease (ESRD) using micro-satellite markers. An interesting polymorphic region in the promotor of the KLK1 gene has recently been identified, where poly-G length polymorphisms are coupled with multiple substitutions. We have sequenced this region in African Americans with and without ESRD. We classified this region into five separate polymorphic loci: four substitution loci and one length locus. The length locus was defined as G repeats starting at position -130 and ending at -121 on the cDNA. We have observed 4 different G repeat alleles ranging from 11.8% for 12 G's to 52.3% for 10 G's in 86 control subjects. Among the four substitution loci, one at position -131, just outside the G repeats, is an A-to-G substitution. The other three polymorphic positions are -129, -128, and -127, all G-to-C substitutions within the G repeats. The C substitution of G's ranges from 2.9% at -127 to 8.2% at -129 in the controls. Association analysis was done using the relative predispositional effect technique. When 92 type 2 diabetic ESRD patients were analyzed against the race-matched controls, no significant association was observed for all five loci. When the first individuals from each of 76 hypertensive ESRD families were pulled together, association for allele 12 of the length locus was detected (allele $P = 0.0044$ and total $P = 0.0189$). When all individuals from each family (107 patients in total) were used in the analysis, even stronger association was observed for this allele (allele $P = 0.0033$, total $P = 0.0099$). Thus, there is evidence that KLK1 has allele association with hypertensive ESRD. Since there was no evidence of association in diabetic ESRD patients, the observed association between KLK1 and hypertension ESRD suggests a link between KLK1 promoter alleles, hypertension, and ESRD.

Using ordinary linkage analysis to detect imprinting in general pedigrees. *E.A. Ludington¹, V.J. Vieland¹, J. Huang²*. 1) Dept Biostatistics, Univ Iowa, Iowa City, IA; 2) Dept Statistics and Actuarial Science, Univ Iowa, Iowa City, IA.

We show here how ordinary linkage analysis can be used to determine whether a disease is imprinted. Simulation studies show that imprinting can be distinguished from either dominant or recessive modes of inheritance in general pedigrees, by comparing the sex-averaged and the sex-specific recombination estimates and maximum lod scores (ZMAX) at a linked locus. If a disease is maternally imprinted, so that offspring are affected only if they receive the disease allele paternally, dominant analyses result in approximately equal male and sex-averaged recombination estimates, and an inflated female estimate, while the sex-specific lod score tends to be less than 50% greater than the sex-averaged lod. By contrast, if the disease is truly dominant or recessive with different sex-specific recombination rates, the sex-averaged recombination estimate is an approximate average of the sex-specific recombination estimates, and the sex-specific ZMAX will be approximately twice the sex-averaged ZMAX. For example, analyzing a rare imprinted disease with 5% recombination, using a dominant analysis with reduced penetrance resulted in a male recombination estimate of 0.03, a sex-averaged estimate of 0.04, and a female estimate of 0.34. Using sex-specific recombination fractions increased the average ZMAX by only 10%, from 7.44 to 8.22. In contrast, analyzing a dominant disease with 5% recombination in male meioses and 50% recombination in female meioses resulted in a male recombination estimate of 0.06, a sex-averaged estimate of 0.24, and a female estimate of 0.43. The sex-specific ZMAX was 2.66, and the sex-averaged ZMAX was 1.25, an increase of over 100%. These patterns appear to hold even when the penetrance of the trait model is misspecified.

Haplotype Linkage Disequilibrium Mapping of Quantitative Trait loci with Phenotypic Selection. *R. Fan*¹, *L. Jin*², *M. Xiong*². 1) Dept of Health Evaluation Sciences, Pennsylvania State University, Hershey, PA; 2) Human Genetics Center, University of Texas-Houston Health Science Center, Houston, Texas.

As the dense map of SNP markers is available in the near future, linkage disequilibrium or joint linkage and linkage disequilibrium analyses will become one of major tools for mapping and identifying quantitative trait loci (QTL). However, since, in general, age of mutations for influencing quantitative trait may be very old, the linkage disequilibrium between the marker and trait loci is unlikely very strong, and hence the power of linkage disequilibrium mapping of QTL may be low. To increase linkage disequilibrium between the marker and trait loci is one of key issue for the success of linkage disequilibrium mapping of QTL. In this report, we propose two strategies for increasing the linkage disequilibrium: phenotypic selection and haplotype linkage disequilibrium mapping. We will develop analytic tools for assessing the impact of phenotypic selection on the linkage disequilibrium under three trait models: single trait locus, two unlinked trait loci, and two linked trait loci. Although the principle and statistical methods for linkage disequilibrium mapping of qualitative trait loci have been well understood and developed, statistical methods for linkage disequilibrium mapping of QTL are less developed. We propose multiple regression methods for linkage disequilibrium mapping of QTL, and demonstrate that the proposed methods are effective in employing phenotypic selection for mapping QTL. Recently, it is increasingly recognized that haplotypes (which can be regarded as a collection of organized markers) may be more powerful than individual, unorganized markers. In this report, we will develop statistical framework for investigation and comparison of the power of multilocus haplotypes and single marker for linkage disequilibrium mapping of QTL. Finally, the proposed methods and strategies will be applied to mapping genes influencing blood pressure.

Linkage and TDT analysis of the CFTR gene in families with atopic asthmatic children. *C. Bombieri¹, R. Galavotti¹, G. Malerba¹, M.C. Lauciello¹, L. Pescolliderung², A.L. Boner³, P.F. Pignatti¹.* 1) Section of Biology & Genetics; Dpt. of Mother & Child, and of Biology-Genetics; Univ. of Verona, Italy; 2) Division of Pediatrics, Hospital of Bolzano; Italy; 3) Section of Pediatrics; Dpt. of Mother & Child, and of Biology-Genetics; Univ. of Verona, Italy.

The genetic components of asthma are largely unknown. Some recent evidence suggests an increase of CFTR gene mutations for asthma patients relative to controls. We have collected 940 subjects from 211 families with atopic asthmatic children from North East Italy. The subjects were characterized for total serum IgE levels, skin prick test (SPT) reactivity to common allergens, bronchial hyperresponsiveness (BHR) to methacholine, and clinical asthma. Multipoint non parametric linkage analysis of the chromosome 7 region where the CFTR gene is located has indicated no suggestive linkage for any phenotype. No significant transmission disequilibrium of 4 RFLP DNA markers (MP6-d9, M470V, 2694 T/G, and J3.11), or of 3 CFTR gene mutations (DF508, G576A, R668C), were observed. A preliminary study in 117 families with affected sib-pairs has shown a trend towards an increased transmission, determined by bootstrap testing (TRANSMIT program), of M470V-2694T/G-J3.11 haplotypes for SPT to animal epithelia or to graminaceae (p values: 0.016 or 0.039, respectively), and of M470V-2694T/G haplotypes for BHR (p=0.03). In conclusion, presently available data indicate no clear evidence for linkage or allele transmission disequilibrium for the CFTR gene in atopic asthma in a North East Italian population.

Comparison of linkage disequilibrium (LD) across long physical distances in four populations with different demographic histories. *F. Durocher¹, A. Dunning¹, C. Healey¹, D. Teare², S. McBride¹, C.F. Xu³, E. Lai⁴, E.*

Dawson⁵, S. Rhodes⁵, I. Purvis³, D. Easton², B. Ponder¹. 1) CRC Dept of Oncology, Strangeways REsearch Lab, University of Cambridge, Cambridge, England; 2) CRC Genetic Epidemiology Unit, Strangeways Research Lab, Cambridge, England; 3) UK Molecular Genetics, GlaxoWellcome, Stevenage, UK; 4) US Discovery Genetics, GlaxoWellcome Inc, USA; 5) Sanger Centre, Cambridge, UK.

In order to test the feasibility of whole genome association studies, we have studied LD between bi-allelic markers from 3 long chromosomal regions: 1400kb on Chr13q12-13, 380kb on Chr19p13.2 and 120kb on Chr22q13.3-ter. In total 38 markers with allele frequencies >0.1 have been examined in 1600 subjects from 4 European populations with different histories: The Afrikaners, Finns, Ashkenazis and East Anglian British. There is remarkably little variation in allele frequency or extent of LD between the different population samples ($F_{ST}=0.0067$). There is no strong evidence for selection of any of the marker alleles as detected by deviation from HWE. We see no clear evidence for recombination hot-spots in any of the chromosomal regions examined. There is a relationship between the proportion of marker-pairs in strong LD (measured by the statistic D') and their distance apart. At <5kb between markers mean D' is 0.67, this declines until at >200kb there is no longer significant LD above the null distribution. D' falls below a mean of 0.30 (the useful value for association studies) at >10kb in all four populations. Thus, this is the maximum distance at which LD between marker-pairs would be strong enough for association studies. The rate of decline of LD with distance is very similar in all four populations, only at the shortest distances (<5kb) is there a significant difference in D' values between the populations. It is therefore apparent that a very high marker spacing (<10kb) will be required for adequate coverage in LD genome scans if it is to have a >50% power of detecting disease loci.

Accurate Allele Frequency Estimation of SNPs Using Pyrosequencing™. *B. Ingemarsson, A. Sylvan, A. Alderborn.*
Pyrosequencing AB, Uppsala, Sweden.

There are several applications in which the ability to determine allele frequencies for single nucleotide polymorphisms (SNPs) would be of great benefit, such as association studies, SNP confirmation and analysis of mutations associated with cancer. The usefulness of SNP analysis in pooled populations of genomic DNA samples will depend greatly on how reliably the smallest difference in allele frequency between two pools can be detected. Pyrosequencing, a novel technique based on real-time sequencing by synthesis detection, provides quantitative sequence data. Therefore, in order to determine the suitability of Pyrosequencing for these types of studies, pools of PCR-products and pools of genomic DNA with different allele frequencies were analyzed. Pyrosequencing showed an excellent linear relationship between peak height and number of nucleotides incorporated, which, in combination with high signal-to-noise ratios for the obtained peaks, facilitated the estimation of allele frequencies even for low frequency SNP alleles. Both pooling strategies gave reproducible and accurate allele frequency estimations, even for an allele frequency as low as 5%. Moreover, Pyrosequencing enabled analysis of both alleles in a single reaction, making the process very reliable and robust. These advantages of Pyrosequencing technology, combined with simple sample handling and the ability to process 96 samples in a single batch, could have a revolutionary impact on the efficiency and cost of analysis for large population studies.

A Multiplex Genotyping and Haplotyping System for SNPs in the IL4R, IL4 and IL13 Genes. *D.B. Mirel, R.L. Reynolds.* Roche Molecular Systems, Alameda, CA.

We have developed a simple and rapid system that permits the simultaneous amplification and genotyping of multiple SNPs in the human IL4R, IL4 and IL13 genes, all candidate loci for inflammatory diseases like asthma. Within the IL4R gene, the assay detects eight coding-region SNPs: I50V, N142N, E375A, L389L, C406R, S478P, Q551R, and S761P. The assay detects two SNPs in the coding region of IL13 [intron 3 and R130Q] and one in the promoter region of IL4 [C-524T]; these three SNPs lie within 20 kb of each other on chr 5q31.

The assay uses a single PCR reaction to amplify nine biotin-labeled products, which are then hybridized under stringent conditions to sequence-specific probes immobilized on a backed nylon membrane strip. The hybridization patterns are then detected by a color development step, and the genotypes read by eye or with scanning software. The assay requires only a thermocycler and a rotating water-bath; up to 40 samples can be genotyped in 2.5 hours. A benefit of this assay is that one subject's genotypes at all loci are collected simultaneously. We have used this assay to genotype a number of ethnic population cohorts; observed allele frequencies agree with published information.

The IL4R gene has the property that many of the cSNPs reside within the 3'-most exon, whose coding region is approximately 1.5 kb. We have exploited this to develop a method for directly haplotyping five of these exon 9 alleles without the need for parental genotypes. Using two allele-specific PCR reactions, we separately amplify the DNA that spans the E375A, L389L, C406R, S478P, and Q551R SNPs from each IL4R chromosome. The alleles on each amplicon are then detected by the same strip hybridization procedure, and the haplotype called directly.

We have confirmed the validity and performance of this haplotyping method on subjects from 3-generation CEPH families, and on other subjects with known haplotypes provided in a blind format. We have haplotyped the IL4R exon 9 SNPs from cohorts of unrelated individuals to examine the validity of computer-generated estimations of haplotype frequencies. We are currently assessing the associations of IL4R haplotypes with asthma.

Haplotype Transmission/Disequilibrium Test (H-TDT) of candidate genes for schizophrenia on chromosome

22q11. *F. Macciardi*¹, *A. Morabito*², *J. Trakalo*¹, *T. Klempan*¹, *A. Ambrosio*¹, *C. Pato*³, *J.L. Kennedy*¹. 1) Dept Psychiatry, CAMH, U of Toronto, Toronto, ON., Canada; 2) Dept of Biostatistics, U of Milan; 3) Dept of Psychiatry, SUNY at Buffalo, NY.

Detecting susceptibility genes for complex traits is difficult given the phenotypic and genetic heterogeneity of these traits. The linkage approach may not yield robust results, having limited power to detect genes that confer a moderate risk to susceptibility. An alternative is the gene-association strategy, where comparisons for genetic polymorphisms are made between patients and controls matched by age, race and sex. Positive associations are thought to result from either true association of the specific polymorphisms with disease, or from the polymorphism studied being in linkage disequilibrium (LD) with a nearby polymorphism or mutation which confers risk. However, many confounding factors hamper the interpretation of these studies and within-family association designs are preferred. The Transmission/Disequilibrium Test is a test for Linkage Disequilibrium (LD) based on the detection of unequal parents-child transmission of high- versus low-risk alleles, a useful alternative for mapping genes with a modest effect on risk. LD mapping using haplotypes is even more powerful than analyzing LD with a single locus, and methods have been proposed for a haplotype TDT strategy (H-TDT, Clayton and Jones, 1999). If LD mapping with haplotypes increases our ability to detect genes in complex disorders, it is essential to preliminarily evaluate the specific LD across any combination of the different polymorphisms at the candidate gene/region. LD mapping would require: (i) evaluating LD between the various polymorphisms across the region, using genotypes from unrelated parents; (ii) testing for the presence of a locus-disease linkage disequilibrium using the TDT for each individual marker, and finally (iii) testing the multiple marker haplotype transmission disequilibrium (H-TDT). Our preliminary analyses in a sample of 104 nuclear families, using two candidate genes (D22S278 and Synapsin III), confirm the feasibility of this approach showing a preferentially transmitted haplotype ($\chi^2 = 8.7$, 1df, $p = .003$).

Identification of SNP markers in the third intron of *GABRB3*. *S. Kim*¹, *D. Gonen*¹, *N. Cox*², *R. Courchesne*³, *S. Pizzo*³, *C. Lord*¹, *B. Leventhal*^{1,5}, *E. Courchesne*^{3,4}, *E. Cook*^{1,5}. 1) Department of Psychiatry, University of Chicago, Chicago, IL; 2) Department of Human Genetics, University of Chicago, Chicago, IL; 3) Children's Hospital, San Diego, CA; 4) University of California, San Diego, CA; 5) Department of Pediatrics, University of Chicago, Chicago, IL.

Previously, linkage disequilibrium between autistic disorder and *GABRB3* 155CA-2 was reported (Cook et al., AJHG, 1998). *GABRB3* 155CA-2 was previously mapped to the third intron, within 10 kb of exons 1a, 1, 2, and 3 of *GABRB3* (Glatt et al., Genomics, 1994). Because of the previous linkage disequilibrium findings of *GABRB3* 155CA-2, we further investigated the proximal portion of the third intron of *GABRB3*. Approximately 7 kb of novel sequence from the third intron that includes *GABRB3* 155CA-2 and *GABRB3* 155CA-1 markers, was determined using a PCR-based genomic walk. Twelve single nucleotide polymorphisms (SNPs) were identified by direct sequencing of PCR products from fifteen probands with autistic disorder. Two of these SNPs, 3.5 kb and 4.5 kb from 155CA-2, were typed in 127 autism trios by SBE-FP (single base extension-fluorescence polarization)(Chen et al., Genome Res., 1999). TDTs for both of these SNPs were non-significant. Other SNPs, including one 6 bp from *GABRB3* 155CA-2 and another 403 bp from *GABRB3* 155CA-2, are currently being typed. These SNPs may be useful for linkage disequilibrium studies of a possible autism susceptibility variant on 15q11-q13.

Empirically determined power calculations for single locus allelic association studies. *R.A. Norman¹, L. Essioux¹, M. Blumenfeld¹, L. Bougueleret¹, A. Cohen¹, I. Chumakov¹, D. Fallin², T. Chu¹, D. Cohen¹, N.J. Schork¹.* 1) Genset, La Jolla, CA; 2) Case Western Reserve University, Dept of Epi-Biostat, Cleveland, OH.

There is a great deal of debate over the utility of dense maps of single nucleotide polymorphisms (SNPs) for association and linkage disequilibrium mapping studies of complex traits and diseases. Much of this debate concerns the likelihood that any of the SNP marker locus alleles typed on individuals in a study will act as good 'surrogates' for functionally-relevant or disease-predisposing alleles at loci residing near SNP marker locus. The degree to which SNP marker alleles will act as good surrogates for neighboring functional variants is dictated by the linkage disequilibrium (LD) strength between the potential surrogate locus alleles and functional alleles. In the absence of knowledge of LD strength, one can assume a specific value for the LD and compute power and sample size guidelines for a prospective study with this LD strength value in mind. However, this approach is assumption-laden and limited to the assumed LD-strength value. In this paper, we propose an alternative strategy for assessing the power of association studies by considering the distribution of likely LD strength values as a function of interlocus distances. This distribution can be estimated empirically from population data. The proposed approach is extremely flexible and is likely to provide an investigator with greater insight into the likely yield of a candidate gene or mapping study than traditional theoretical or simulation-based studies. We offer a number of examples of our approach, comparing the power of potential mapping studies that assume that samples are taken from different ethnic groups sampled within the United States or from greater France. We also discuss the limitations of our proposed approach as well as possible extensions.

Transmission disequilibrium test (TDT) for polymorphisms of alcohol dehydrogenases 3 and 4 using alcoholics and non-alcoholics. *J.K. McClintick¹, H.J. Edenberg^{1,2}, T. Foroud¹*. 1) Medical and Molecular Genetics, Indiana University, Indianapolis, IN; 2) Biochemistry and Molecular Biology, Indiana University, Indianapolis, IN.

In certain populations, a functional polymorphism of alcohol dehydrogenase 2 (*ADH2*) has been associated with a protective effect against alcoholism. *ADH2* polymorphisms are rarely found in populations of European ancestry. Functional polymorphisms of alcohol dehydrogenases 3 and 4 (*ADH3*, *ADH4*) do occur at significant rates among this population. *ADH3* and *ADH4* are located in close proximity on chromosome 4. We wanted to determine if any haplotype of these 2 genes is associated with alcoholism or its absence. We performed a transmission disequilibrium test using individuals and their parents from a group of American families selected for a large scale study of alcoholism, the Collaborative Study on the Genetics of Alcoholism. Two groups of individuals were selected that had been diagnosed as alcoholics by 2 different criteria and another 3 groups of individuals were selected that had been determined to be unaffected by an increasingly restrictive measure of symptoms. None of these groups had significant differences in the haplotypes that were transmitted from their parents.

Analysis of Candidate Genes for Susceptibility to Conotruncal Cardiac Defects. *J.C. Yang⁴, L. Mitchell^{1,3,4}, J.A. Campanile⁴, H.M. Russell⁴, K. Hoess⁴, M. Kenton⁴, E. Goldmuntz^{1,4}, D.A. Driscoll^{1,2,4}.* 1) Departments of Pediatrics; 2) Obstetrics and Gynecology; 3) Biostatistics and Epidemiology, University of Pennsylvania; 4) Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia.

The etiology of conotruncal cardiac defects (CTCD) is heterogeneous and includes chromosome abnormalities (aneuploidy, 22q11.2 deletions), single gene disorders, maternal diabetes, and teratogenic exposures. However, in the majority of affected individuals a specific etiologic agent(s) cannot be identified. The cause of CTCD in such patients is thought to involve multiple genetic and environmental risk factors. Although animal studies support the involvement of multiple genes in the development of the conotruncus and outflow tract, relatively little is known about the genes that may influence the development of these structures in humans. We are using a candidate gene approach to identify genes which influence susceptibility to CTCD in humans. For each candidate gene, at least one intragenic polymorphism is selected and tested for association with CTCD using the transmission disequilibrium test (TDT). To date, ninety-nine families, consisting of a proband with CTCD and unaffected parents, have been genotyped for markers at eight candidate loci (MSX1, MTHFR, TGFA, TGFB3, EDN1, PAX3, NTF3 and NF1). Proband with aneuploidy or a 22q11.2 deletion were excluded from this study. Preliminary data for the MSX1 gene suggests that genetic variation at this locus may be associated with an increased risk of CTCD ($P = 0.03$). Analysis of the results at the other seven loci are predominantly negative, however, these loci cannot be excluded as risk factors because of the small sample size. These loci, as well as new candidate genes, are currently being investigated with additional families and markers.

Linkage disequilibrium mapping in a 1 Mb region of 9q34 using different populations. *C.L. Willoughby, M.T. Rebello, S.J. Jeremiah, M.W. Burley, M. Agochiya, L. Davies, J. Sinclair, J. Mallet, J. Wolfe, S. Povey.* Department of Biology, The Galton Laboratory, University College London, Wolfson House, 4 Stephenson Way, London UK.

Devising efficient strategies for the study of complex disorders requires an understanding of the distribution of recombination and linkage disequilibrium across different parts of the genome and in different populations. In a pilot study we looked at 218 grandparental chromosomes haplotyped from the CEPH families, taken from a core 32 families and removing those individuals known to be related, for a region of approximately 1 Mb between *TSC1* and D9S66 in 9q34. This region has been almost completely sequenced. As part of a larger study of chromosome 9 we have identified all the recombinant chromosomes in these families and over this 2.5 cM distance there have been 7 recombinant events in males and 7 in females, out of 554 meioses examined. Recombination immediately proximal to this region is higher in females, and immediately distal is higher in males.

The 11 markers examined in the region include SNPs in *ABO*, *TSC1* and *SURF1*, and eight microsatellites. In addition a group of markers within the *ASS* gene (3 cM proximal) and three on 9p, were examined as controls. LD between markers was examined using Fisher's exact test. No significant LD was found between markers in the region and the control markers. Within the region highly significant LD was found across one region of 400 kb but was much less across two other regions of 50 kb and 100 kb. Most directly observed recombinants lie in the regions of least disequilibrium. Although numbers are small these data suggest the recombination events may be unevenly distributed across this 1 Mb region and that LD across such distances may be largely determined by recombination.

We are now examining a number of the critical markers in other populations. Preliminary results on over 80 haplotypes from a UK population ascertained through alpha-1-antitrypsin deficiency offers some support for the above observations, and further studies are in progress.

Haplotype analysis in male patients with the complete form of congenital stationary night blindness (CSNB1) minimizes the CSNB1 region to about 2.1 cM. *K. Pesch¹, J. Tomiuk², E. Zrenner³, M. Broghammer¹, W. Berger⁴, A. Meindl⁵, B. Wissinger¹, F.K. Jacobi¹, C.M. Pusch¹.* 1) Molecular Genetics Laboratory, University Eye Hospital, Tuebingen, Germany; 2) Institute of Anthropology and Human Genetics, Tuebingen, Germany; 3) University Eye Hospital, Tuebingen, Germany; 4) Max-Planck-Institute for Molecular Genetics, Berlin, Germany; 5) Department of Medical Genetics, Ludwig-Maximilian-University, Munich, Germany.

Congenital stationary night blindness (CSNB1) is a hereditary retinal disease associated with myopia, decreased visual acuity, and nystagmus. X-linked CSNB can be differentiated into a complete (CSNB1) and an incomplete (CSNB2) form. Whereas the causative CSNB2 gene has been recently identified, the CSNB1 locus has so far only been genetically linked to the region Xp11.3-Xp11.4. In order to refine the CSNB1 consensus interval between the proximal marker DXS228 and the distal marker DXS556 (i.e. 3-5 cM), we performed haplotype analysis on 23 unrelated male patients with a complete CSNB phenotype, and 50 unaffected probands as control group. The allelic variation of 10 highly polymorphic markers (DXS556, DXS8042, DXS1368, DXS574, DXS993, DXS8012, DXS1207, DXS1201, DXS8085, DXS228) was studied. The allelic similarity of haplotypes among patients was significant (p smaller than 0.05) whereas non-significant within the control group. Furthermore, such differences between unaffected probands and patients could also be found for a reduced set of five loci DXS993, DXS8012, DXS1207, DXS1201, and DXS8085; and thereby reducing the critical CSNB1 interval to about 2.1 cM.

Refined mapping of the Shwachman-Diamond syndrome locus at 7p12-q11. *M. Popovic*^{1,2}, *S.L. Goobie*^{1,2}, *J. Morrison*², *G.R.B. Boocock*^{1,2}, *N. Ehtesham*², *L. Ellis*^{3,4}, *P. Durie*^{3,4,5}, *J.M. Rommens*^{1,2}. 1) Department of Molecular and Medical Genetics, University of Toronto, Toronto, ON, Canada; 2) Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, ON, Canada; 3) Program in Integrative Biology, The Hospital for Sick Children, Toronto, ON, Canada; 4) Division of Gastroenterology and Nutrition, The Hospital for Sick Children, Toronto, ON, Canada; 5) Department of Pediatrics, University of Toronto, Toronto, ON, Canada.

Shwachman-Diamond syndrome (MIM260400) is a rare autosomal recessive disorder presenting in infancy with failure to thrive and recurrent infections. The primary clinical manifestations are exocrine pancreatic insufficiency, hematological dysfunction and skeletal abnormalities, although a broad and variable phenotypic spectrum may be present. Serious infections, bone marrow failure and leukemia are primary concerns for morbidity. The basic biochemical defect is unknown. Linkage analysis in affected families has identified a major disease locus at 7p12-q11. Critical recombination events observed in multiplex families narrowed the locus to a 6 cM interval flanked by D7S1830 and D7S1839. Further refinement of the critical interval has been accomplished by haplotype analysis. Densely spaced markers used in haplotype construction were ordered by radiation hybrid and STS content mapping. Comparison of extended haplotypes in 34 affected families revealed existence of 5 ethnic group-specific disease haplotypes. Region of overlap between these 5 haplotypes refined the disease locus to a 2.7 cM interval. Detailed physical and transcript mapping of the minimal interval and characterization of positional candidate genes are underway to identify the disease gene.

Family based association analysis of folate genes in OFC reveals possible role of MTHFR. *N. Prescott, J. Tyson, D. Kelberman, M.M. Lees, R.M. Winter, S. Malcolm.* Department of Clinical and Molecular Genetics, Institute of Child Health, London, UK.

Orofacial clefting (OFC) is an epithelial fusion defect which occurs during the first few weeks of pregnancy. This congenital anomaly is influenced by genetic factors as demonstrated by an elevated sibling relative risk of 30-40. Despite this marked familial segregation, the underlying genetic etiology of OFC is likely to be complex and heterogeneous and influenced by environmental disturbances. Studies have suggested that supplementary folic acid during pregnancy may decrease the chance of an affected OFC child, in those known to be at increased familial risk, by 25-65%. A recent whole genome scan in OFC affected sibling pairs highlighted two genomic regions which harbour genes involved in folate metabolism, i.e., MTHFR (1p36.3) encoding Methylene tetrahydrofolate Reductase and FOLR1 (11p13) folate receptor gene. Using a resource of 250 affected trio/sibling pair pedigrees we have examined sequence variations in these genes via RFLP analysis and TDT to determine a possible association between two common SNPs and OFC. We have been unable to demonstrate Linkage Disequilibrium (LD) between the MTHFR 677T variant, known to reduce enzyme activity and plasma folate levels in homozygotes, and OFC. However, we have observed a significant LD between OFC and the MTHFR 1298A polymorphism ($p < 0.05$), which does not effect enzyme activity, accompanied by a marked distortion from Hardy-Weinberg equilibrium in this region ($p < 0.001$). Haplotype analysis has revealed an over-transmission of the 677T/1298A haplotype to OFC individuals with an under-transmission of the rare 677T/1298C haplotype. The latter involves the occurrence of the two variant alleles in cis which have not been observed in similar studies of this nature. These findings confirm linkage to 1p36 and implicate MTHFR as having a possible role in OFC etiology.

A genome wide search for genetic risk factors in Icelandic stroke patients. *S. Gretarsdottir¹, H. Jonsson¹, A. Kong^{1,2}, M. Frigge¹, H. Gudjonsdottir¹, O. Einarsson¹, S. Jonsdottir¹, S.Th. Reynisdottir¹, S.M. Bjarnadottir¹, Th. Gudmundsdottir¹, J. Sainz¹, S. Sveinbjornsdottir³, G. Einarsson³, F. Jakobsson⁴, E.M. Valdimarsson⁴, U. Agnarsson⁵, G. Thorgeirsson⁵, V. Gudnason⁵, K. Stefansson¹, J. Gulcher¹.* 1) deCODE Genetics, Reykjavik; 2) Department of Human Genetics and Statistics, University of Chicago; 3) University Hospital-Hringbraut, Reykjavik, Iceland; 4) University Hospital-Grensas, Reykjavik, Iceland; 5) Icelandic Heart Association Heart Preventive Clinic, Reykjavik, Iceland.

Stroke is a complex disease consisting of a group of heterogeneous disorders with multiple genetic and environmental factors. The clinical phenotype is complex but has traditionally been divided into multiple subtypes of ischemic and hemorrhagic stroke. In our study we used a broad but rigorous definition of the stroke phenotype including hemorrhagic stroke, ischemic stroke and transient ischemic attack. Our extensive population based genealogy database was used to cluster stroke patients from a list of 2500 patients diagnosed with stroke over a 5 year period at the University Hospital in Reykjavik. Patients with stroke due to Icelandic hereditary cystatin C amyloid angiopathy were excluded. Patients were then selected for the study based on this genealogical fractionation. Nearly 500 stroke patients and 400 relatives, connected within 179 pedigrees, were genotyped using 900 microsatellite markers. The data were analysed using affecteds-only, non-parametric, allele sharing methods. A locus was found that met the accepted criteria for genome wide significance. This locus does not correspond to known susceptibility loci for stroke or its secondary risk factors. Due to suggestive marker order problems in the publicly available maps we physically and genetically mapped this locus. The results of the genome wide search and mapping will be presented.

Results of logistic regression modeling support linkage at chromosome 4p16-15 in European American pedigrees multiplex for systemic lupus erythematosus. *C. Gray-McGuire^{1,2}, H. Yu¹, P. Gaffney³, K.L. Moser^{1,2}, J. Kelly¹, B.R. Neas¹, J. Olson², K.B. Jacobs², T. Behrens³, J.B. Harley¹.* 1) Dept Arthritis & Immunology, Oklahoma Medical Research Fndn, Oklahoma City, OK; 2) Case Western Reserve University, Cleveland, OH; 3) University of Minnesota, Minneapolis, MN.

Systemic lupus erythematosus (SLE) is a complex autoimmune disorder involving at least hormonal, environmental, and genetic factors. High heritability, high monozygotic twin concordance rates, familial aggregation, association with candidate genes, and the results of five genome scans demonstrate support for a genetic component. Several candidate linkage regions have been identified by the various scans, including 1q22-24 (lod=3.37), 2q37 (lod=4.24), and 6p21-11 (lod=4.16), but few have been supported by independent studies. We present evidence and support, from two independent data collections, for an effect at 4p16-15 in European Americans (EA). This effect was first identified (lod=2.18) using 55 European American families multiplex for SLE, a subset of the present Oklahoma collection. An analysis of the expanded Oklahoma collection (297 EA sibpairs and 42 EA affected relative pairs) using the revised multipoint Haseman-Elston regression technique for concordant and discordant sib pairs (SIBPAL2) and a conditional logistic regression technique for affected relative pairs (ARP) agreed with the original finding ($p=0.0003$; and lod=3.84). Analysis of an independent collection of 153 EA affected sibpairs, 6 EA affected half-sibpairs, and 13 EA affected relative pairs collected through the University of Minnesota using the methods described above support a linkage effect in this region (lod=1.7). Fine-mapping studies are currently underway to isolate and potentially characterize one of the first candidate linkage regions to be supported at this magnitude in independent studies.

Suggestive Linkage of Large Artery Compliance to Chromosome 2 and 18 in African Americans: The HyperGEN Study. *D.K. Arnett¹, R.B. Devereux², A.T. Dewan¹, A. Oberman³, M.A. Province⁴, D.C. Rao⁴.* 1) Dept Epidemiology, Univ Minnesota, Minneapolis, MN; 2) Weill Medical College of Cornell University, New York, NY; 3) University of Alabama at Birmingham, Birmingham, AL; 4) Washington University School of Medicine, St. Louis, MO.

Structural and functional alterations in large arteries occur in hypertension, which result in reductions in arterial compliance. Recent evidence suggests variation in candidate genes contribute to reductions in arterial compliance. However, linkage of genomic regions to this complex phenotype is not yet reported. As part of the NHLBI Family Blood Pressure Program - Hypertension Genetic Epidemiology Network, we tested whether genomic regions linked to echocardiographic measures of aortic compliance. We examined African American hypertensive sibships (n=159) recruited in North Carolina and Alabama and collected echocardiograms that were centrally read. Compliance was determined by dividing pulse pressure by stroke volume (mean = $0.7 + 0.24\text{mmHg/ml}$). Pulse pressure was defined as the difference in systolic and diastolic blood pressure taken at the time of the echocardiographic examination, and stroke volume was determined from the echocardiogram. All siblings were hypertensive, 88% reported antihypertensive medication use and the mean age was 52 years. Microsatellite markers (n=387), approximately equally spaced throughout the genome, were typed by the NHLBI Mammalian Genotyping Service. Arterial compliance was adjusted first for age, age², field center, and heart rate within sex-race groups (P1), and additionally for systolic blood pressure, lean body mass, antihypertensive medication use, diabetes, smoking, and ventricular wall thickness (P2). The search for quantitative trait loci for arterial compliance was carried out using a multipoint model-free variance components method implemented in GENEHUNTER. Suggestive linkage was identified for P1 on chromosome 2 (LOD = 2.15, 231 cM from the pter) and for P2 on chromosome 18 (LOD = 1.76, 43cM from the pter). These data suggest that there may be influential genetic regions contributing to aortic compliance in African American sibships ascertained for hypertension.

Effect of covariates on quantitative trait linkage analysis. *L.D. Atwood¹, D.M. Grabrick², A.F. Wilson³*. 1) Division of Epidemiology & Institute of Human Genetics, Univ Minnesota, Minneapolis, MN; 2) Health Sciences Research, Mayo Clinic, Rochester, MN; 3) Genometrics Section, IDRB, NHGRI, NIH, Baltimore, MD.

It is widely accepted that allowing for the effects of an environmental covariate will improve the power to detect linkage of a quantitative trait. However, few studies have quantified the effect of a covariate on power or location estimates. We addressed this problem by performing a linkage analysis of a simulated quantitative trait affected by two loci, each of which accounts for 15% of the total trait variation. We examined 10 combinations of the two loci in which the minor allele was either recessive or dominant and had frequency of 0.1 or 0.3. For each combination, there were five levels of an environmental covariate accounting for 0% to 60% (in increments of 15%) of the total trait variation. For each of the 50 locus-covariate combinations, G.A.S.P. was used to simulate 100 replications of 100 nuclear families of size 5. Each locus was placed at 45cM on separate 90cM chromosomes. Each chromosome contained 10 evenly spaced four-allele markers each with heterozygosity 0.75. A variance-components approach, as implemented in GENEHUNTER, was used to test for linkage. Power was estimated by the average of the maximum lodscore on each chromosome. Error was estimated by the average of the distance from the maximum lodscore to the correct location.

Results showed that power increased and error decreased in a non-linear fashion as the strength of the covariate increased. Average lodscores ranged from 0.7 (covariate 0%) to 5.6 (covariate 60%). Average error ranged from 23cM (covariate 0%) to 8cM (covariate 60%). Power was maximized when both loci were recessive with frequency 0.1 and minimized when both loci were dominant with frequency 0.3. Surprisingly, error showed the opposite relationship. Error was minimized when both loci were dominant with frequency 0.3 and maximized when both loci were recessive with frequency 0.1.

These results indicate that a covariate correction can increase power in a non-linear fashion. Also, error in the location estimate is a function of both the covariate and the genetic model.

Genetic heterogeneity of autosomal recessive hereditary cataract. *G.D. Billingsley^{1,2}, M.K. Priston^{1,2}, F.L. Munier^{3,4}, A. Balmer⁴, D.F. Schorderet³, A. Verner⁵, M. Fraser⁵, T. Hudson⁵, E. Héon^{1,2}.* 1) Dept Ophthalmology, Vision Science Research Program, University of Toronto; 2) The Hospital for Sick Children Research Institute; Toronto, Ontario, Canada; 3) Unit of Oculogenetics and Division of Medical Genetics; CHUV; 4) Hôpital Ophthalmique Jules Gonin; Lausanne, Switzerland; 5) Montreal Genome Centre, McGill University Health Centre, Montreal, Quebec, Canada.

Cataracts constitute the leading cause of blindness worldwide and the mechanisms of lens opacification remain unclear. The genetics of this condition are complex and all three forms of Mendelian inheritance have been reported. To date at least 15 loci for autosomal dominant and one for autosomal recessive cataract have been identified across the human genome. We have studied a large 3 generation European family affected with a unique autosomal recessive central and cortical pulverulent cataract. It is characterised by an epinuclear dustlike opacification to a variable degree involving the anterior and posterior cortex. Progressive increase in the density of the nuclear and cortical opacities as well as premature nuclear sclerosis necessitates surgery by the age of forty. A total of 8 affected from one generation, 31 unaffected and 8 spouses were characterized clinically and studied by linkage analysis to identify the location of the underlying genetic defect. Two-point and multipoint linkage analysis was done using 17 candidate loci including the 3p21 autosomal recessive locus, related to congenital cataract and proteins involved in lens structure, development or metabolism. Linkage was excluded with these loci encompassing over 384 cM or 11% of the genome. Currently a genome-wide screen is being undertaken. The molecular characterization of this phenotype may shed light on the complex cascade of events modulating lens differentiation and lens opacification.

Resolution of marker order and distance to refine linkage in specific reading disability. *A.M. Brower¹, K.E. Deffenbacher¹, R.K. Olson², J.C. DeFries², S.D. Smith¹.* 1) University of Nebraska Med Ctr, Omaha, NE; 2) Institute for Behavioral Genetics, University of Colorado.

Linkage analysis by our group and two others have localized a gene influencing specific reading disability (dyslexia) to the short arm of chromosome 6. The locus size varies between the three groups from 11cM spanning D6S422 (pter) through D6S291 (Fisher et al., AJHG, 64:146, 1999), and 10cM spanning D6S109 through D6S306 (Grigorenko et al., AJHG, 60:27, 1997), to 5cM spanning D6S461 through D6S258 (Gayan et al., AJHG, 64:157, 1999). The order and genetic distance in cM of the markers used in linkage analysis also varies depending on the method used to generate the maps, either family studies or radiation hybrids. To refine this localization and correlate the various maps, we have constructed an integrated marker map of this region using draft and finished sequence data available from the Sanger Center. Results of the sequence analysis of the genetic markers spanning this interval from 6p21.1-23 were able to resolve ambiguities between existing maps and provided a correlation between genetic distance in cM and physical distance in Mb. With the exception of D6S109, we were able to locate each marker as well as the corresponding parent sequence in either draft or finished sequence data. We then utilized the BLAST homology search program to locate each finished clone containing individual markers to the larger contigs of contiguous, assembled sequence in the chromosome 6 ACEDB database at the Sanger Center and in the LocusLink database at NCBI. We present an integrated map for the dyslexia locus with comparisons of the genetic distances in Kosambi cM generated by the Marshfield map maker program and the Genethon map in cM, genetic distances in cR generated by various radiation hybrid maps, and the physical distances generated by our sequence map in Mb. Multipoint linkage analysis with reading disability using the Haseman-Elston algorithm in GENEHUNTER2 confirmed and refined the linkage previously reported in a subset of this population with a smaller group of markers (Gayan et al., 1999). Funded by NIH-NICHD HD27802.

Genetic Linkage and Linkage Disequilibrium at Chromosome 1q41-42 in Human Systemic Lupus

Erythematosus. *P.M. Gaffney¹, R.R. Graham¹, W.A. Ortmann¹, S.A. Selby¹, E.C. Baechler¹, K.B. Shark¹, T.C. Ockenden¹, K.E. Rohlf¹, N.L. Walgrave¹, C. Langefeld², S. Rich², T.W. Behrens¹.* 1) Department of Medicine, University of Minnesota, Minneapolis, MN; 2) Department of Public Health Sciences, Wake Forest University School of Medicine.

Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease of unknown etiology with a strong genetic component. Three genome screens and a targeted study have found evidence for genetic linkage at D1S229 in the 1q41-42 region. This region, which lies just centromeric to the poly ADP ribose polymerase (PARP) gene, is syntenic to the SLE1d locus recently identified in the NZM2410 murine model of SLE. In order to further characterize this region in our family collection, twelve microsatellite markers flanking D1S229 were genotyped in 211 SLE sib-pair families and 123 SLE trio families. The best evidence for linkage was found at D1S2616 (LOD=1.15) using multipoint non-parametric analyses with GENEHUNTER PLUS. A two marker multi-allele transmission distortion test (TDT) revealed evidence for LD between specific alleles of markers D1S245-D1S425 ($p<0.0027$), D1S425-D1S2827 ($p<0.008$), D1S229-D1S227 ($p<0.018$), D1S227-D1S2616 ($p<0.00097$), and D1S2616-D1S2641 ($p<0.025$). A three marker haplotype (D1S229-D1S227-D1S2616) spanning this region demonstrated significant transmission distortion in a proportion of our Caucasian SLE families ($p<0.000096$). These results support and extend previously published results demonstrating linkage of the SLE phenotype to 1q41-42. The presence of genetic linkage, the potential synteny of this region with a murine SLE locus, and the evidence for LD warrant further gene discovery efforts in the 1q41-42 interval.

Genetic Dissection of Human Systemic Lupus Erythematosus: Identification of Linkage Disequilibrium in the Class II/III Region of the HLA. *R.R. Graham¹, W.A. Ortmann¹, S.A. Selby¹, E.C. Baechler¹, K.B. Shark¹, T.C.*

Ockenden¹, K.E. Rohlf¹, N.L. Walgrave¹, J.T. Elder², R. Nair², P. Gregersen³, P.M. Gaffney¹, T.W. Behrens¹. 1) Department of Medicine, University of Minnesota, Minneapolis, MN; 2) Department of Dermatology, University of Michigan, Ann Arbor, MI; 3) Department of Genetics, North Shore University, Long Island, NY.

Systemic Lupus Erythematosus (SLE) is a chronic, debilitating autoimmune disease caused by a complex interaction of genetic and environmental factors. Association studies targeting HLA-DQ alleles and TNF- α alleles have implicated the HLA (6p21) as an important genetic factor in SLE. Supporting these findings, three independent genome screens have found suggestive evidence for genetic linkage in the 6p21 region. Recent fine mapping of the 6p21 region using 211 SLE sib-pair families revealed strong evidence for genetic linkage (LOD score of 3.30) within the HLA. However, due to a high density of genes and extensive linkage disequilibrium across the HLA it has proven difficult to identify relevant susceptibility genes. Therefore, the HLA is being genetically dissected using a dense map of 43 known and several newly developed polymorphic microsatellite markers distributed across the length of the region. Analysis thus far of 16 markers genotyped in 334 SLE families using the transmission disequilibrium test (TDT) has identified two intervals showing strong evidence for linkage disequilibrium in SLE. Interval 1 is ~360,000 base pairs near the Class II-Class III boundary and is centered on M6s137 (Allele 4: TDT $p < 0.00011$) [see AJHG 66:1833-1844, 2000 for marker designation]. Interval 2 is ~350,000 base pairs near the Class III-Class I boundary and is centered on M6s125 (Allele 16: TDT $p < 0.0093$). These studies have identified several apparent high-risk HLA marker haplotypes in SLE. Additional marker analyses within these families should allow a further narrowing of the susceptibility intervals using a combination of TDT and ancestral recombinant haplotype analyses.

Linkage analysis in a large Brazilian family maps a gene for autosomal recessive (AR) craniometaphyseal dysplasia to chromosome 6q and confirms genetic heterogeneity for AR spondylocostal dysplasia. *P. Iughetti¹, L.G. Alonso², N. Alonso³, M.R. Passos-Bueno¹*. 1) Departamento de Biologia, Universidade de São Paulo, São Paulo, São Paulo, Brazil; 2) Departamento de Morfologia, Escola Paulista de Medicina, São Paulo, São Paulo, Brazil; 3) Departamento de Cirurgia Plástica, Universidade de São Paulo, São Paulo, Brazil.

Craniometaphyseal dysplasia (CMD), a genetically heterogeneous group of skeletal disorders, is characterized by sclerosis of the skull base, vault and facial bones, and deformity of the metaphyses of the tubular bones. The majority of CMD cases presents an autosomal dominant (AD) pattern of inheritance with only a few families suggesting an autosomal recessive (AR) mode of transmission. A locus for the AD form has recently been mapped to chromosome 5p, while the locus for the AR type remains to be located. We performed here genomewide scanning by homozygosity mapping in a large Caucasian inbred AR CMD family with 5 living affected individuals. Significant lod-scores were obtained with markers from the 6q21-22 region. Based on the homozygous region shared by the affected patients and identification of two recombinant individuals, we defined a 7 cM critical interval for this disease gene, between D6S302 and D6S1639. We have also excluded COL10A1, the most promising positional candidate, as being the responsible for this disorder. These findings, besides confirming that AD and AR CMD are two distinct entities, will be of fundamental importance for the identification of the gene associated with AR CMD, which might be involved in osteoblast differentiation. Interestingly, there are two individuals from this genealogy with clinical features compatible with a form of AR nonsyndromic spondylocostal dysplasia (SD). SD is an heterogeneous group of disorders, both with AR and AD forms. A gene for an AR SD type has been mapped to 19q13 region, however, we did not find evidence of linkage between this 19q region and the skeletal disease segregating in our family, thus suggesting genetic heterogeneity for AR SD. Supported by FAPESP, HHMI, CNPq, PRONEX.

Genetic linkages found in 126 pedigrees multiplex for systemic lupus erythematosus. *J. Kelly¹, K.L. Moser², C. Gray-McGuire¹, S. Rao², J. Salmon³, R.P. Kimberly⁴, J.C. Edberg⁴, B.R. Neas^{1,5}, J.M. Olson², J.B. Harley^{1,5}.* 1) Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) Case Western Reserve University, Cleveland, OH; 3) Hospital for Special Surgery, New York, NY; 4) University of Alabama at Birmingham, Birmingham, AL; 5) University of Oklahoma, Oklahoma City, OK.

We have evaluated 126 pedigrees multiplex for systemic lupus erythematosus containing 698 family members in an effort to better define the genetics of this systemic autoimmune disease. Multiple genome scans with >300 markers demonstrate ~20 suspected linkages. Of these, several are of sufficient magnitude or also found in other studies to conclude that a susceptibility gene has a high likelihood of being present. Using the criteria ($\text{lod} > 3.3$), significant evidence for linkage is found at 1q22-23, $\text{lod} = 3.97$ and 3.43 for Fc gamma RIIA and D1s2762, respectively, by maximum-likelihood model-based methods. Affected relative pair evaluation (S.A.G.E. 4.0) has revealed linkage at 4p16-15 with D4s2366 ($\text{lod} = 3.62$). Additional genome scan analyses of both univariate SLE related traits and derived Principal Components revealed multiple signals. The three largest multivariate effects obtained when using the Principal Component analysis were at 7p13 with D7s1818 ($p = 0.003$), 4q36.1 with D4s1652 ($p = 0.020$) and 15q15.1 with D15s659 ($p = 0.027$). When univariate SLE related traits were analyzed, the largest effect, influenced by immunological characteristics, was found at 2q34 with D2s1384 ($p = 0.00048$). For the univariate principal components, the most significant linkage result was at 4q36 with D4s1652 ($p = 0.00007$). The genes responsible for these linkages are not known. These data establish that there are several linkages that each represents a gene or genes that have a role in immunopathogenesis and contribute toward developing dysregulation of the immune system causing lupus.

Combinatorial Mismatch Scanning (CMS) detects Identity by Descent (IBD) in small samples of probands and controls from the same Mendelian breeding units. *S.C. Heath*¹, *R. Robledo*^{2,3}, *W. Beggs*², *G. Feola*³, *C. Parodo*³, *A. Rinaldi*^{3,4}, *L. Contu*⁵, *M. Siniscalco*^{2,3}. 1) Dept of Human Gen, Memorial Sloan-Kettering Cancer Ctr, New York, NY; 2) Coriell Institute for Medical Research, Camden, New Jersey; 3) Sardinian Center for Studies of Genome Diversity, University of Cagliari, Italy; 4) Chair of General Biology, Faculty of Odontoiatry, University of Cagliari, Italy; 5) Chair of Medical Genetics, University of Cagliari, Italy.

Severe myopia of dominant type, a mono-factorial disorder recently mapped to 18p11.31, is commonly spread in the genetically isolated village of Carloforte (Sardinia, Italy) and thus suitable to test the efficiency of the CMS procedure to detect identity by descent (IBD) among probands and controls from the same breeding Mendelian unit. Accordingly, 16 myopic probands and 35 normal controls were selected for being proven descendants from the original founder group and for not having ancestors in common at least up to the grandparental generation. DNAs were genotyped for thirteen markers of autosome 18 separated from each other by a genetic distance of about 10 cM. Allelic distributions were similar at all loci in probands and controls with the sole marked exception of the candidate marker D18S63. The significance of this result was estimated by using a bootstrap technique, which allowed the testing of multiple aspects of the dataset in a robust and efficient manner. This was used to estimate the probability of the sample under the null hypothesis of no difference in frequency distribution between affected and control individuals. Locus D18S63 was found to have the smallest sample probability ($p < 0.002$), which is significant at the 0.05% level after correcting for testing multiple loci. This result underlines the efficiency of the CMS to detect IBD out of small numbers of probands and controls when both are part of well defined breeding Mendelian units. The requirements needed to use the methodology in the absence of information on candidate loci and for its extension to complex traits and diseases are discussed.

Homozygosity mapping of ataxia-oculomotor apraxia locus 1 to chromosome 9 and evidence for genetic

heterogeneity. *M.C. Moreira*^{1,2}, *C. Barbot*^{2,3}, *P. Mendonça*², *C. Miranda*², *P. Coutinho*^{2,4}, *J. Sequeiros*², *M. Koenig*¹.
1) IGBMC - CNRS, INSERM, ULP - Strasbourg, France; 2) UnIGENe - IBMC, ICBAS Univ Porto - Porto, Portugal; 3) Hospital de Crianças Maria Pia - Porto, Portugal; 4) Hospital Sao Sebastiao - Sta. Maria da Feira, Portugal.

The hereditary ataxias are a heterogenous group of diseases representing 10% of the nervous system genetic disorders. Ataxia-oculomotor apraxia (AOA), a new autosomal recessive syndrome, seems to have a higher frequency in Portugal. AOA patients present cerebellar ataxia, oculomotor apraxia, neuropathy and dystonia. Although AOA is a late-onset *Ataxia-telangiectasia (AT)*-like disease, it could be differentiated from AT by the absence of telangiectasias, chromosomal breakage, tendencies for malignancy and frequent infections, normal immunoglobulins, and AFT levels, and normal T- and B-lymphocyte markers, as well as normal chromosomes 7 and 14. As a consequence of the survey of hereditary ataxias and spastic paraplegias that is being done in Portugal since 1993 (covering a global population of more than 4 millions inhabitants) a total of 115 families with recessive ataxias has been identified. Among these, Friedreich ataxia (FRDA) is the most frequent (61 living patients/41 families), followed by AOA (24 living patients/14 families homogeneously distributed over the country). We began the AOA linkage studies with a whole genome screen with 382 polymorphic markers in the 5 most informative AOA Portuguese families (3 consanguineous families and 2 large families with unknown consanguinity). Our results suggested linkage to chromosome 9 for 3 of the 5 families. Further analysis using additional markers and 6 new families (3 consanguineous families and 3 families with unknown consanguinity) confirmed the linkage for 6 families in a 6 cM region on chromosome 9. Homozygosity and linkage disequilibrium was used to demonstrate homogeneity among at least 5 of these 6 families. The 5 remaining families were excluded for linkage at this region, indicating the existence of genetic heterogeneity among AOA families. The largest of these 5 families is under investigation for the identification of a second AOA locus.

The LD-LOD score Analysis for Detecting Linkage Adaptive to Linkage Disequilibrium. *Y. Jiang, J. Huang.*

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Due to rapid advancements in molecular technology, it is becoming feasible in the near future to use a highly dense map of genetic markers, such as the single-nucleotide polymorphisms (SNPs) or markers with multiple alleles, in a genome screen for mapping disease genes. With a dense map, some of the markers could be in linkage disequilibrium (LD) with the disease-predisposing alleles. Significant LD may also result from other factors such as admixture of two or more subpopulations differing in allele frequencies. It is useful to incorporate LD in linkage studies because it can increase the statistical power. However, in practice, the extent of LD between a marker and disease is usually unknown. Therefore, it is of interest to develop linkage methods that are adaptive to LD. We consider a modified LOD score method for linkage detection that is adaptive to LD (LD-LOD). This LD-LOD method can use multiple markers in a multipoint analysis and can be naturally applied to general pedigrees. We show that the LD-LOD score method efficiently combines two sources of information: (a) the IBD sharing scores which are informative for linkage regardless of the existence of LD; and (b) the contrast between allele-specific IBD sharing scores which are informative for linkage due to the presence of LD. For example, for affected sib-pair (ASP) data, a simple recessive LD-LOD score is an adaptive combination of the TDT and the ASP mean test. We demonstrate that the LD-LOD score method has relatively good statistical efficiency in comparison with the ASP mean test and the TDT for a broad range of LD and genetic models. Therefore, the LD-LOD score method is an useful approach for detecting linkage when the extent of LD is unknown.

The gene for autosomal dominant Limb-Girdle Muscular Dystrophy and Paget Disease of Bone in a large family maps to a unique locus on 9p22.3-q12. *M.J. Kovach¹, V.E. Kimonis¹, S. Leal², B. Waggoner¹, A. Salam², R. Khadori³, D. Gelber⁴.* 1) Dept Pediatrics, Southern Illinois Univ Sch Med, Springfield, IL; 2) Lab Statistical Genetics, Rockefeller University, NY, NY; 3) Dept Internal Medicine, Southern Illinois Univ Sch Med, Springfield, IL; 4) DeptNeurology, Southern Illinois Univ Sch Med, Springfield, IL.

The limb-girdle muscular dystrophies (LGMDs) encompass a large family of clinically and genetically heterogeneous neuromuscular disorders. We have identified a large family with autosomal dominant LGMD and Paget disease of bone (PDB) with Alzheimer disease in two individuals. Muscle biopsy shows non-specific changes and vacuolar myopathy in the oldest male. The existence of these two pathologically distinct phenotypes co-segregating among affected individuals within a single family is a rare occurrence. To date, only three other families with a similar phenotype have been reported.

Molecular studies have previously identified 4 chromosomal loci for autosomal dominant LGMD and 2 loci for autosomal dominant PDB. Preliminary linkage studies have excluded these loci, leading to a hypothesis that a unique gene is involved in the pathogenesis of the disease in this family. A genome-wide scan of 39 family members including 9 affected individuals, indicated linkage to chromosome 9p21-q21 with marker D9S301 (max LOD=3.64). Haplotype analysis with a high density of markers flanking D9S301 mapped the disease locus to a 30.94 cM region on chromosome 9p22.3-q12 flanked by markers D9S1869 and D9S1118. The autosomal recessive locus for vacuolar myopathy, IBM2, which maps within 1 cM of D9S1791 may be within the critical region, however recombinations distal to D9S1118 exclude the LGMD2H locus mapped to chromosome 9q. Linkage analysis of additional families is being performed towards narrowing the critical region for LGMD/PDB.

Immunocytochemistry of muscle biopsy material with antibodies specific for components of the dystrophin-sarcoglycan complex showed a complete absence of staining for a-sarcoglycan in affected individuals.

A genome wide screen for allele sharing in the first 300 sibling pairs of the NARAC collection. *D. Jawaheer^{1,4}, M.F. Seldin^{2,4}, C.I. Amos^{3,4}, W. Chen^{3,4}, J. Monteiro^{1,4}, L. Criswell⁴, S. Albani⁴, L. Nelson⁴, D.O. Clegg⁴, R. Pope⁴, H.W. Schroeder⁴, S.L. Bridges⁴, D.S. Pisetsky⁴, D. Kastner⁴, R. Wilder⁴, T. Pincus⁴, L. Callahan⁴, P.K. Gregersen^{1,4}.* 1) Biol & Human Genet., North Shore Univ Hosp, Manhasset, NY; 2) UC Davis, Davis, CA; 3) MD Anderson Cancer Center, Houston, TX; 4) The North American Rheumatoid Arthritis Consortium.

We have completed a genome-wide screen of 263 multiplex RA families containing 309 affected sibling pairs, using 378 microsatellite markers (Marshfield). In addition to meeting ACR criteria for RA, entry into the study was restricted to sib pairs in whom at least one sibling exhibited erosions on hand X-rays. Overall, 82% were seropositive, and mean disease duration was 17 years. Mean age of disease onset was 38. An initial non-parametric linkage analysis was performed using SIBPAL. As expected, significant sharing at the HLA region was observed (0.56, $p < 0.00001$). More interestingly, several regions outside the HLA region showed evidence of linkage ($p < 0.005$): D1S235, D4S1647, D5S816, D8S277, D8S373, D12S398, D12S373, D14S1280, D16S403 and D17S1301. The strongest evidence for linkage outside of the HLA region was with marker D4S1647, with sharing of 0.56, $p < 0.001$. Several of these regions overlap with chromosomal locations implicated in other autoimmune diseases. D16S403 lies within 15 cM of a region which has been implicated in lupus, psoriasis and inflammatory bowel disease. The D1S235 marker at 1q41 has been linked to lupus. The D12S373 marker lies in a region previously implicated in RA, and is within 5 cM of TNFR1, a plausible candidate for RA susceptibility. These data are consistent with the hypothesis that genes in the HLA region play a major role in RA susceptibility, but that numerous other regions contribute lower levels of genetic risk. Confirmation of these findings will require replication in a second, larger dataset of RA sibling pairs, as well as further association studies. The NARAC has ascertained over 800 RA sibling pairs, and recruitment is in progress; these families will provide an opportunity to extend the initial findings reported here.

Genome-wide screen for quantitative trait loci (QTLs) influencing lipid levels in a founder population. D.L.

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Cardiovascular disease is a major health problem worldwide and elevated lipid levels are well-known risk factors. We conducted a genome-wide search for QTLs for LDL, HDL, Lp(a), and triglyceride levels using 563 marker loci (average 6 cM map) in the Hutterites, a founder population of European ancestry that practices a communal, farming lifestyle. The 522 adult (>14 years) Hutterites in our study are descendants of 64 ancestors and are related to each other in a 13-generation, 1,623-member genealogy. The small number of founding genomes, their relatively recent founding, and communal lifestyle (including a uniform diet) should facilitate the search for loci influencing complex traits. Linkage analysis for QTLs was conducted using a variance component approach, developed for mapping in inbred pedigrees (Abney et al, 1999, *Am J Hum Genet* 65:A240). Locus-specific and genome-wide significances were assessed by simulation. Two marker loci reached genome-wide significance: D6S305 (at 166 cM) with Lp(a) and D2S410 (at 125 cM) with triglycerides. D6S205 is approximately 1 cM from the Lp(a) structural gene, which is known to have a major influence on Lp(a) levels. Three additional loci showed suggestive evidence for linkage: D18S843 (at 28 cM) with Lp(a) ($P=0.0001$), IFNA (at 36 cM on chr. 9) with triglycerides ($P=0.0002$), and D19S433 (at 52 cM) with LDL ($P=0.001$). The most significant linkages with HDL ($P=0.002$) were with D20S901 (at 26 cM) and D5S1986 (at 45 cM). [cM distances from p-ter based on the Marshfield map] These data suggest that Lp(a), triglycerides, and perhaps LDL levels may be influenced by genes with relatively major effects, and that the communal and relatively uniform Hutterite lifestyle and diet may enhance the effects of QTLs on lipid levels.

A gene for Pelger Huët anomaly maps to chromosome 1q41-43. *K. Hoffmann*^{1,2}, *H. Karl*³, *R. Kaps*³, *D. Mueller*⁴, *G. Nuernberg*¹, *A. Reis*¹, *K. Sperling*⁵. 1) Gene Mapping Center, Max Delbrueck Center for Molecular Medicine, Berlin; 2) Franz Volhard Clinic at the Charité, Humboldt University, Berlin; 3) General practitioners, Chemnitz and Gelsenau; 4) Department of Pediatrics, Klinikum Chemnitz; 5) Department of Medical Genetics, Charité at the Humboldt University, Berlin, Germany.

Purpose: The Pelger Huët anomaly is an autosomal dominant benign disorder altering granulocyte morphology. The prevalence varies from country to country, but usually affects about 1 in 5000 individuals. However, we found a small town in south eastern Germany where the frequency is much higher and feasible for identification of a Pelger Huët locus by linkage analysis.

Methods: Light microscopy screening of blood smears from 4386 individuals revealed one homozygote and 53 heterozygote probands with Pelger Huët anomaly in the village Gelsenau (total number of inhabitants 6530). From that pool, 49 persons from 9 obviously unrelated families have been selected for genetic analysis. The sample includes one homozygote and 30 heterozygote affected individuals. A total genome scan with 389 microsatellite markers was performed followed by fine mapping and multipoint parametric linkage analysis.

Results: Region 1q41-43 showed evidence for linkage to Pelger Huët anomaly with a lod score of 5.2. Combined linkage and haplotype data confirmed the critical region between markers D1S229 and D1S2847 (241.6 cM to 247.6). A shared trait haplotype could be identified.

Conclusion: The increased frequency in the village Gelsenau seems to be due to a founder effect by a shared common ancestor. Cloning the gene might give insight to altered cell development processes that are also involved in other disorders. Acquired Pelger like granulocyte morphology is observed in some forms of cancer (e.g. leukemia), infections (e.g. tuberculosis, infectious mononucleosis) and intoxications (e.g. long term valproate therapy).

A family based association test for quantitative traits with pedigrees. *O. Mokliatchouk*. Department of Mathematics and Computer Science, Drexel University, Philadelphia, PA.

Family based tests of association are currently developed for categorical, quantitative and censored traits with nuclear families. These tests can be applied to data on pedigrees, but more efficient methods that take into account familial correlations are of interest. The proposed test is an extension of a family based association test for data on quantitative traits with pedigrees. The test involves conditioning on the minimal sufficient statistic for association in the absence of linkage in order to avoid confounding caused by factors other than linkage, and estimating the conditional distribution of the quantitative trait given the observed marker genotypes and unobserved trait genotypes. In the case of known frequencies of trait genotypes, such estimation is obtained using the results from the theory of mixture models. For the case of unknown trait genotype frequencies, a new theory is proposed. Results of simulations that explore the performance of the proposed tests are described. The procedures are illustrated through an analysis of data on APOE genotype, a mutation of alpha-2 macroglobulin gene (A2M) and Alzheimer's disease.

Localization of the gene for a novel disease characterized by Intestinal Lymphangiectasia to 6p21-22. Evidence for genetic heterogeneity. *M. Kambouris^{1, 2}, S. Shabib¹, H. Nazer¹, A. Al-Mehaidib¹, S. Abu-Amero¹, B.F. Meyer¹.* 1) King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia; 2) Yale University School of Medicine.

Intestinal lymphangiectasia is the dilatation of the intestinal lymphatic system vessels and is characterized by protein losing enteropathy, steatorrhea and lymphopenia. Four consanguineous (all first cousin marriages) unrelated nuclear families affected with a novel autosomal recessive disorder characterized by intestinal lymphangiectasia were identified. Disease onset is early (~2 years) and clinical features include lymphedema, protein losing enteropathy, malabsorption, growth failure, generalized edema, diarrhea and recurrent chest and skin infections. Disorders characterized by hereditary intestinal lymphangiectasia and/or lymphedema have been described but none seems to correlate with the inheritance pattern and clinical symptomatology seen in these families. Additionally, no genes have been identified causing autosomal recessive intestinal lymphangiectasia or lymphedema. Homozygosity mapping with 440 microsatellite markers spaced at approximately 10 cM intervals was performed in these four families. For family 1 (four affecteds), a maximum LOD score of 4.3 at $\theta=0$ was obtained for two consecutive markers (GGAA15B08, D6S1017) identifying an area of homozygosity by descent thus localizing the disease gene to 6p21-22. A maximum linkage interval of 30 KcM was defined by the presence of recombinants at the distal GATA163B10 and heterozygosity at the proximal GATA11E02. The unique phenotype and localization to 6p21-22 are consistent with the identification of a novel disorder. Linkage to the 6p21-22 region could not be established for the other three families indicating genetic heterogeneity. Affected members of families 2 (three affecteds) and 3 (two affecteds) share an area of homozygosity in chromosome 13 which if inherited by descent would result in a LOD score > 3 . Linkage to 6p21-22 or the candidate region of chromosome 13 could not be demonstrated for family 4 (two affecteds). Fine mapping of the linkage intervals for families 2 and 3 and further genotyping of family 4 are in progress.

The use of summed maximum lods as a simple and approximate measure of evidence for linkage based on multiple independent data sets. *J. Huang¹, K. Wang², V.J. Vieland²*. 1) Statistics & Actuarial Sci, Univ of Iowa, Iowa City, IA; 2) Biostatistics, Univ of Iowa, Iowa City, IA.

For complex disorders, it usually requires multiple data sets to achieve definitive evidence of linkage, raising the question of how best to measure linkage evidence across multiple independent data sets. One approach is to pool all the data sets and calculate a single summary statistic at each locus, e.g., a heterogeneity lod (HLOD; Smith, 1961). This procedure essentially measures the average evidence (HLOD-A) across the data sets, which might seem like a good method for eliminating any false positive evidence arisen through sampling variability in the smaller individual data sets. However, this procedure may be overly conservative because it averages out any strong linkage signal with the weak ones. An alternative is to sum maximum HLODs across all the data sets (HLOD-S), which might seem problematic because of the potentially high degrees of freedom. In spite of these considerations, however, we show that for ASP data, in the presence of inter-sample heterogeneity HLOD-A tends to underestimate linkage evidence; while HLOD-S provides a good approximation to a correct LR statistic allowing for inter-sample heterogeneity, with better power than HLOD-A. Asymptotic results and simulations show that the HLOD-S accumulates just over 1 d.f. per data set, even in realistic sample sizes ($N=100$ ASPs). We also show that the distribution of HLOD-S is asymptotically the same as the distribution of the sum of maximum MLS statistics [Risch, 1990; Holmans, 1993]. Extensions to other forms of the likelihood and to larger, more informative pedigree structures remain to be explored. Nevertheless, these results show that when multiple studies yield different results at the same locus, summing the individual maximum lods rather than averaging them can provide a better overview of the true overall strength of evidence.

Genome wide scan of bipolar disorder and evidence of parent of origin effect. *M.G. McInnis¹, T.H. Lan¹, A.T. Mahoney¹, J. Chellis¹, F.J. MacMahon², D.F. MacKinnon¹, J.B. Potash¹, H. Chen¹, S.G. Simpson¹, T.H. Beaty¹, J.R. DePaulo¹.* 1) Johns Hopkins University, Baltimore, MD; 2) University of Chicago, Chicago IL.

Sixty-five multiplex bipolar pedigrees ascertained through a treated BPI proband and two affected first degree relatives were genotyped at on average 10 cM intervals across the genome. The sample consists of 573 typed subjects, 129 were diagnosed with bipolar I (BPI), 102 with bipolar II (BPII), 78 with recurrent unipolar depression (RUP), and 8 with schizoaffective-manic (SA-M). We analyzed our data as a whole group and partitioned according to the sex of the affected parent, there were 26 "paternal" and 34 "maternal" pedigrees, analytic methods included multipoint parametric and nonparametric analyses (GENEHUNTER) and IBD analysis of affected sib pair (SIBPAL and simIBD). The data are suggestive of a genome wide parent of origin effect. Our analyses were consistent with our previously reported evidence of a susceptibility locus on 18q21, with a maximum NPL 2.5 ($p=0.008$) and HLOD of 1.8 in those 65 pedigrees. Among the 23 paternal pedigrees, the maximum NPL was 3.2 ($p=0.001$) and HLOD was 1.4 on chromosome 18. A locus on 8q24 yielded an NPL of 3.2 ($p=0.0008$), HLOD=2.1 was primarily due to the maternal pedigrees. Including RUP in the analysis identified a region on 13q13 with an NPL of 2.5 ($p=0.008$) and HLOD = 3.0 due to the maternal pedigrees. A slightly smaller NPL of 2.2 ($p=0.01$) and HLOD of 1.9 was observed at 2p13 among paternal pedigrees. Analysis using ASPEX identified the 8q24, 13p13, and 18q21 regions with MLS greater than 2.0 with further suggestive evidence of heterogeneity among maternal and paternal pedigrees. In the total sample 16 markers showed excess allele sharing (p less than 0.01); 6 of these showed support from flanking markers. SimIBD identified 6 markers with evidence (empirical p greater than 0.01) of increased allele sharing among affected sib pairs. The two-point linkage analyses using FASTLINK identified 14 LOD scores 1.0 or higher under a dominant model (the highest of which was 3.1 at D8S256) and 17 LODs 1.0 or higher under the recessive model (highest 2.7 at D12S82).

X-LINKED CLEFT PALATE AND ANKYLOGLOSSIA: REFINEMENT OF THE MINIMAL CRITICAL REGION IN Xq21.3. *A.C.B. Marcano^{1, 2}, J.E. Ming¹, Y.Z. Du¹, R.A. George¹, S.G. Ryan¹, A. Richieri-Costa², M.*

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Cleft palate is among the most common congenital anomalies. In humans, secondary cleft palate occurs during the seventh to ninth week of embryological development when the bilateral palatine shelves fail to fuse. While family recurrence data suggests the involvement of genetic factors in isolated cleft palate, a Mendelian pattern of inheritance is not usually observed. Rather, the multifactorial threshold theory is postulated for most cases of isolated cleft palate, but neither the genetic nor the environmental components are well understood. However, in a few families, the segregation of cleft palate suggests a major genetic determinant. There are reports of a few families exhibiting autosomal dominant inheritance of isolated cleft palate. X-linked cleft palate has been documented in some families. In these families, some of the affected males and carrier females presented with ankyloglossia. Linkage studies have assigned the gene for X-linked for cleft palate and ankyloglossia (CPX) to an approximately 2.0 Mb region on Xq21.3-q22 (reference). We report two large unrelated Brazilian kindreds with X-linked cleft palate and ankyloglossia. In one kindred, there are 5 affected individuals or obligate carriers, and in the other there are 13 individuals carrying the abnormal gene. We performed linkage analysis on these kindreds, and our data were consistent with the previously reported region. We were able to narrow the critical region, and the region is flanked by the markers DXS8109 and DXS1217. This is the first report of this condition in the Brazilian population, and our linkage studies have narrowed the critical region and may aid in identification of the gene.

Genome-wide Search for the Ossification of the Posterior Longitudinal Ligament. *K. IKARI^{1,2}, K. Furushima^{1,2}, S. Maeda^{1,3}, H. Koga³, S. Komiya³, S. Harata², I. Inoue¹*. 1) Lab. of Genetic Diagnosis, IMS, Univ. of Tokyo, Minato-ku, Tokyo, Japan; 2) Dept. of Orthopedic Surgery, Hirosaki Univ., Hirosaki, Aomori, Japan; 3) Dept. of Orthopedic Surgery, Kagoshima Univ., Kagoshima, Kagoshima, Japan.

Ossification of the posterior longitudinal ligament of the spine (OPLL) is characterized by ectopic ossification in the spinal ligaments leading to a various degree of myelopathy by a compression of the spinal cord. OPLL is commonly observed among Japanese and throughout other Asian populations. The incidence of OPLL in the general Japanese population was reported to be 1.9 - 4.3% over 30 years of age. Although its etiology is thought to involve a multiplicity of factors, epidemiological and family studies strongly implicate genetic susceptibility in the pathogenesis of OPLL. The disease has a substantial genetic component, a risk in siblings compared to general population risk (ls) of 10. Previously, we have reported of suggestive evidence of linkage to a candidate gene, collagen 11A2, where only a candidate region (HLA region at 6p21.3) was tested for linkage. To define the genetic causalities of OPLL in more extensive manner, we performed a genome-wide scan with 138 affected sib-pairs. Non-parametric linkage analysis was performed with affected sib-pairs by the use of two different programs. Identical by descent (IBD) was tested for possible linkage with SIBPAL from S.A.G.E. package (single point analysis). Multipoint affected sib-pairs linkage analysis was performed using GENEHUNTER. We found that five principal loci of possible linkage were identified on chromosome 1, 6, 13, 16, 21 ($p < 0.01$). And the most significant evidence of linkage was observed with D21S263 at chromosome 21q ($P=0.000009$). Multipoint linkage analysis revealed that the peak linkage was also located close to D21S263 (maximum lod score = 3.4). These mapping results could be an important step towards identifying susceptible genes for OPLL.

Two candidate genes for polycystic ovary syndrome (PCOS); follistatin and the insulin receptor. *M. Urbanek*¹, *K.R. Vickery*¹, *R.S. Legro*², *D.A. Driscoll*³, *J.F. Strauss*³, *A. Dunaif*⁴, *R.S. Spielman*¹. 1) Department of Genetics, University of Pennsylvania, Philadelphia, PA; 2) Department of Obstetrics and Gynecology, Pennsylvania State University, Hershey, PA; 3) Center for Research on Reproduction and Women's Health and Department of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA; 4) Division of Women's Health, Brigham and Women's Hospital, Boston, MA.

PCOS is a common endocrine disorder characterized by hyperandrogenism, chronic anovulation and insulin resistance. In an initial screen of 37 candidate genes for PCOS (PNAS 96:8573-8578), we identified two promising genes, follistatin (FST) and the insulin receptor (INSR). Here we describe these regions in more detail.

In the FST gene we identified 17 variant sites in 85 individuals from 19 multiplex families. Sixteen variants were too rare to make a major contribution to PCOS susceptibility. The only common variant, a single base-pair change in the 3' UTR, had only very weak evidence for association with PCOS in our 314 trios ($c^2=4.04$). There was no evidence for linkage in the 44 new affected sib pairs (ASPs) tested. These results suggest that contributions to the etiology of PCOS from FST are likely to be small.

For the INSR region we studied nine polymorphic markers flanking INSR. In ASPs maximum identity by descent (IBD) was observed at D19S884 (IBD=0.63; $c^2=8.11$). Allele 8 of D19S884 showed significant evidence (TDT $c^2=15.85$) for disequilibrium for a PCOS susceptibility gene in the region. However, D19S884 maps 1-2 cM centromeric to INSR, and linkage disequilibrium is usually maintained only over much smaller regions. Therefore, the allele 8 association may be due a gene other than INSR. To resolve this question we are 1) determining the physical map distance between INSR and D19S884, and 2) testing as potential PCOS candidate genes all known genes and any open reading frames that are located near D19S884.

Examination of five large Essential Tremor families for linkage to known loci. *E. Spiteri*¹, *D. Doheny*², *D. de Leon*³, *D. Mirel*⁴, *K. Wilhelmsen*⁴, *S.B. Bressman*³, *M.F. Brin*², *C. Falk*⁵, *B.E. Morrow*¹. 1) Albert Einstein Col. of Med., Bronx, NY; 2) The Mount Sinai Medical Center, NY, NY; 3) Beth Israel Medical Center, NY, NY; 4) University of California, San Francisco, San Francisco, CA; 5) New York Blood Center, NY, NY.

Essential tremor is the most common movement disorder in humans affecting 0.3 to 1.7% of the population across every ethnic group. Familial Essential Tremor (FET) is generally a monosymptomatic disorder manifesting itself in an action tremor which primarily affects the upper limbs but can also affect the legs, head, voice and trunk. Although the tremor may be progressive, it is not known to reflect a neurodegenerative disorder. Pedigree studies suggest that FET is inherited in an autosomal dominant manner. Previous studies have demonstrated linkage between FET and marker loci on chromosomes 2p22-2p25 and 3q13. A third study suggested linkage to chromosome 4p. Thus, FET appears to be genetically heterogeneous. Our goal is to understand the molecular basis of FET and how it relates to normal neurological function. Five large multigenerational FET families have been collected, consisting of 171 individuals, 45 of whom are affected. Three families are of German descent, one of Italian/German descent and one of English/Scottish descent. All patients have the typical expression of FET with tremor in the arms and a variable pattern in other body parts. Power analysis generated ELODS of between 1.2 and 3.3 for each of the families. We examined the families for linkage in the three regions mentioned above, using genetic markers spanning 10-20 cM in each region. Dominant inheritance with incomplete penetrance was assumed. Two-point analysis was carried out with MLINK. Multilocus analysis using affecteds only was performed with Genehunter. All five families showed evidence against linkage in the regions studied, and the multilocus analysis excluded all three regions in these five families. Exclusion of these three regions in these families suggests additional loci for FET, further increasing the evidence that FET is genetically heterogeneous. These families will be used for a genome-wide scan to search for other loci.

Mapping refinement and mutation analysis of syndromic X-linked high myopia. *T.L. Young, S.M. Ronan, A.B. Alvear, J.M. Peterson, A.T. Dewan, A.M. Holleschau, R.A. King.* Depts. of Ophthalmology and Genetics, Univ. of Minnesota, Minneapolis, MN.

Purpose: The Bornholm eye disease (BED), described in one family in 1988, consists of X-linked high myopia, high cylinder, optic nerve hypoplasia, reduced electroretinographic flicker with abnormal photopic responses, and questionable deuteranopia. The disease has been mapped to chromosome Xq28 (MYP1 gene locus). We now describe a second family, also of Danish descent, that originates from the nearby islands of Mn and Zealand. Affected individuals had mean increased high myopia (-13.18 D), high cylinder (1.81 D), and axial length (28.39 mm). All affected males had tilted optic nerve heads with temporal crescents. All had subnormal photopic electroretinogram results. Color vision testing revealed protanopia, however. Linkage analysis and candidate region mutation screening were performed. **Methods:** X chromosome genotyping, fine-point mapping, and haplotype analysis of the DNA from 22 individuals (8 affected males, 5 carrier females) was performed using 23 polymorphic microsatellite markers. Cytogenetic analysis, and DNA sequencing of the locus control region (LCR) and of the red-green pigment gene array coding regions was performed. **Results:** Significant maximum lod scores of 3.38 and 3.11 at $\theta = 0.0$ were obtained with markers DXS8106 (band Xq27.3) and DXYS154 (telomeric end of band Xq28), respectively. Haplotype analysis defined an interval of 34.4 cM. High-resolution cytogenetic analysis of two affected males showed no deletion abnormalities. Sequencing revealed no mutations in the LCR, and a red-green hybrid gene upstream of three normal green pigment genes in the cone pigment gene array. **Conclusion:** This kindred phenotypically resembles that described for the BED, and supports chromosome Xq27.3-q28 mapping of the MYP1 locus. Phenotypic features of this kindred also overlap with other types of retinal cone dystrophies that map to Xq27 and Xq28. Mutation screening results in our family ruled out matches with those forms of cone dysfunction, however. The BED and this phenotype may be similar disorders, and may represent a newly described X-linked cone dystrophy.

Ultero-mutilating peripheral neuropathies: genetic linkage study and mutation analysis of candidate genes. K.

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Inherited peripheral neuropathies complicated by mutilating neuropathic ulcers sometimes necessitate amputation of distal parts of a limb. Linkage analyses in families with this phenotype have shown genetic heterogeneity and two autosomal dominant loci have been identified so far: Charcot-Marie-Tooth type 2B (CMT2B) on chromosome 3q13-q22 and Hereditary Sensory Neuropathy type I (HSN I) on 9q22. We studied four large families with ultero-mutilating neuropathy and detailed clinical and neurophysiological studies were performed in order to make genotype-phenotype correlations. One of these families confirms the existence of a CMT2B locus located on 3q13-q22, and supports the notion that CMT2B is clinically and electrophysiologically homogeneous disorder with prominent distal muscle weakness and wasting and ultero-mutilating features. Recombination events in affected individuals reduce the CMT2B candidate gene interval considerably from 25 to 10 cM between the flanking markers D3S1589 and D3S1549. Two candidate genes located within the CMT2B region have been screened for disease causing mutations at the cDNA and genomic level.

Fine-Mapping the Familial Focal Segmental Glomerulosclerosis Locus on Chromosome 11q - Search for the Genetic Mutation. *M.P. Winn¹, P.J. Conlon², K.L. Lynn³, D.N. Howell¹, A.H. Smith³, C.S. Alexander¹, F.L. Graham¹, L.D. Quarles¹, M.A. Pericak-Vance¹, J.M. Vance¹.* 1) Dept Med/Div Nephrology, Duke Univ Medical Ctr, Durham, NC; 2) Nephrology, Beaumont Hospital, Dublin, Ireland; 3) Nephrology, Christchurch Hospital, Christchurch, New Zealand.

We have previously described a locus for autosomal dominant focal segmental glomerulosclerosis (FSGS) which maps to an 8 cM region on chromosome 11q21-22 in a large family from New Zealand. The disease in this family is characterized by variable age of onset (16-61 years of age), severe nephrotic syndrome, early progression to end-stage renal disease (ESRD) and no recurrence after renal transplantation.

Subsequent fine-mapping using polymorphic microsatellite markers has identified recombination events at D11S1343 and D11S1394 reducing the candidate region to approximately 3.5 cM. Candidate genes in the region include a family of matrix metalloproteinases (which cleave different types of collagen), inhibitors of apoptosis genes 1 and 2 (apoptotic suppressors) and radixin (binds the barbed end of actin filaments to the plasma membrane). Preliminary analysis of several metalloproteinases using a combination of Southern blot analysis, SSCP, HPLC and direct sequencing of exons as well as intron/exon boundaries has revealed evidence of polymorphisms that are common to affected and control individuals.

In summary, the area on chromosome 11q21-22 linked to autosomal dominant FSGS has been narrowed to approximately 3.5 cM. Candidate gene analysis is ongoing but has yet failed to identify the causative mutation in this disease.

Assignment of the gene responsible for chronic recurrent multifocal osteomyelitis and congenital dyserythropoietic anemia to the short arm of chromosome 18 and examination of TGIF as a candidate gene. *M.K. Tayeh⁴, H.A. Majeed², N. Al-Masri³, H. Al-Rimawi¹, A. Pelet⁴, A. Munnich⁴, S. Lyonnet⁴, H. El-Shanti^{1,4}.* 1) Dept. of Pediatrics, Jordan University of Science & Technology, Irbid, Jordan; 2) Dept. of Pediatrics, University of Jordan, Amman, Jordan; 3) Dept. of Pathology, Jordan University of Science & Technology, Irbid, Jordan; 4) Medical Genetics, INSERM U393, Hopital Necker-Enfants Malades, Paris, France.

Chronic recurrent multifocal osteomyelitis (CRMO, MIM259680) is an inflammatory bone disease of unknown etiology characterized by bone pain and fever, with an unpredictable course of exacerbation and spontaneous remissions. This disorder has been reported in sporadic cases although the literature contains few familial cases. A spontaneously occurring mutation in the mouse causing tail kinks and limb deformity is thought to be an animal model for CRMO (cmo). The cmo locus maps to mouse chromosome 18. On the other hand, congenital dyserythropoietic anemia (CDA) is a pathologic alteration of the morphology and function of RBCs resulting in increased phagocytosis of the abnormal cells by bone marrow macrophages. Our group has described a syndrome with the combination of CRMO and CDA in 4 individuals from one extended inbred family from Jordan. The pattern of inheritance of this syndrome in this family was assumed to be autosomal recessive due to the presence of multiple affected sibs, of both sexes, in each of two sibships, normal parents and consanguinity. We report here, the localization of the gene responsible for this syndrome to a 5.5 cM interval on the short arm of chromosome 18. TGIF, a gene encoding a transcription factor, which acts as a repressor of transcription in the presence of Smad2, maps to the same region of linkage on chromosome 18p. Heterozygous mutations in TGIF homeodomain have been found in holoprosencephaly patients. By virtue of its function and position, we suspected that mutations in the Smad2 binding site of TGIF might explain the CRMO/CDA syndrome in our family. We did not identify any mutations in the entire coding sequence of TGIF in our patients, thus excluding this candidate gene for this complex disorder.

A quasi-likelihood method for allele frequency estimation. *X. Wu^{1,2}, C. Ober², M.S. McPeck¹*. 1) Dept Statistics, Univ Chicago, Chicago, IL; 2) Dept Human Genetics, Univ Chicago Chicago, IL.

Correctly estimating allele frequency is critical for gene mapping studies. For simple codominant markers and samples of unrelated individuals, allele-frequency estimation of a particular allele in the population can be easily carried out by calculating the proportion of the number of this allele to the total number of alleles in the sample. The results of this naïve counting method are the maximum-likelihood estimates (MLEs) of allele frequencies. For estimating allele frequency using family data, Boehnke described a method maximizing the likelihood of the family data in the framework of pedigree analysis. However, calculating the full likelihood of a complicated pedigree, such as in Hutterites, is computationally very difficult, if not impossible. Here we described a quasi-likelihood method for allele frequency estimation as an approximation of MLE in complicated pedigrees. For most mapping studies using large inbred populations, the naïve counting method was chosen for its simplicity and lack of an alternative method. To compare our quasi-likelihood method with the naïve counting method, we performed simulations in the Hutterite pedigree for calculating allele frequencies in the Hutterite founder population. Both methods gave unbiased estimate of allele frequencies in our study. However, the variance of allele frequencies from the quasi-likelihood method is consistently smaller than that from the naïve counting method for a range of different allele frequencies. Simulation studies are under way to investigate how these two methods will affect the estimation of other statistics, such as conditional expected HBD (EHBD), in the Hutterites.

Determining the Expected Value and Confidence Interval for the Number of Genotype Incompatibilities in a Family. *A.D. Skol, M. Boehnke.* Dept Biostatistics, Univ Michigan, Ann Arbor, MI.

It is relatively easy to discern between genotyping errors (or mutations), incorrect parentage, and mislabeling of a sample once genotype data are available upon completion of a genome scan. However, genotype data often becomes available for only one or two chromosomes at a time. With this amount of data, it is often difficult to distinguish incorrect parentage or other pedigree error from genotyping error or mutation. In addition, it is generally difficult to determine the correct relationship between individuals when parentage has been incorrectly reported and at least one parent is not genotyped. Because of our poor ability to determine what type of error created the incompatibility, more useful questions at this point in the mapping project may be: how many incompatibilities do we expect to see and how likely are our observed results, given our prior beliefs or estimates of the various error probabilities. An unusually large number of incompatibilities may suggest that additional measures to assure the fidelity of the data need to be put in place.

We have developed a method and will write software to calculate the expected value and variance of the number of incompatibilities in a nuclear family given a set of parameters that describe the error probabilities, allele frequencies, number of markers genotyped, and pattern of genotyped individuals. From this, an approximate confidence interval of the number of inconsistencies may be calculated to determine whether the observed number of incompatibilities is consistent with the assumed parameter values. It is a natural extension to calculate the expected odds that a given incompatibility pattern is due to one type of error versus another; for example the odds of false paternity versus genotyping error. We also investigate how the probability of detecting an error changes with number of loci and individuals genotyped. We will attempt to extend this work to complete pedigrees.

Mapping of suppressor gene for dermatitis of NOA (Naruto Research Institute Otsuka Atrichia) mice, an animal model of allergic dermatitis. *M. Tamari*¹, *O. Watanabe*², *Y. Onouchi*², *Y. Shiimoto*³, *I. Hiraoka*³, *I. Inoue*¹, *Y.*

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To investigate the genetic factors involving in pathogenesis of atopic dermatitis, we used NOA mouse, an animal model for atopic or allergic dermatitis. Although a large number of genetic studies have been performed, genes predisposing to or developing these diseases are still not well understood because of the etiological complexity in humans. The NOA (Naruto Research Institute Otsuka Atrichia) mouse is an animal model of allergic or atopic dermatitis, a condition characterized by ulcerative skin lesions with accumulation of mast cells and increased serum IgE. Previously, we reported that a major gene responsible for dermatitis of NOA mouse lied on the middle of chromosome 14. The mode of inheritance was autosomal recessive with incomplete penetrance. Furthermore, we searched a modifier gene responsible for the NOA phenotype. Finally, we found two candidate loci for suppressor genes for atopic or allergic dermatitis on the middle of chromosome 7 and telomere of chromosome 13 ($c2=14.66$, $P=0.00013$ for D7Mit62, and $c2=15.342$, $P=0.000089$ for D13Mit 147).

The acrodermatitis enteropathica gene maps to chromosome 8q24. *K. Wang¹, E.W. Pugh², S. Griffen¹, K.F. Doheny², W.Z. Mostafa³, H. El-Shanti⁴, J. Gitschier¹.* 1) Department of Medicine and Pediatrics, HHMI, University of California, San Francisco, CA; 2) Center for Inherited Disease Research, Johns Hopkins University, Baltimore, MD; 3) Department of Dermatology, Faculty of Medicine, Cairo University, Cairo, Egypt; 4) Department of Pediatrics and Medical Genetics, Jordan University of Science and Technology, Irbid, Jordan.

Acrodermatitis enteropathica (AE) is a rare autosomal recessive pediatric disease of zinc deficiency characterized by dermatitis, diarrhea, alopecia and growth failure. The disease, which results from insufficient uptake of zinc by the intestine, can be fatal unless zinc is supplemented in the diet. To map the AE gene, a genome-wide screen was performed in a consanguineous Jordanian family with five affected individuals. Seventeen individuals including four affecteds were genotyped with a set of 364 markers with an average interval of 9 cM. We compared the number of alleles present for unaffected sibs, affected sibs and parents. Three markers, D8S373, D10S212, and D6S1021 had a pattern consistent with tight linkage to a recessive disease: one allele for the affected sibs and multiple alleles in the other groups. Two point parametric linkage analysis using FASTLINK identified one region with a maximum LOD score above 1.5 (1.94 at D8S373 $q=0.001$). Fourteen additional markers flanking D8S373 were then typed in the family including additional family members to fine-map the AE gene by use of haplotype analysis. All five affected individuals were found to be homozygous for a common haplotype spanning approximately 3.5 cM on chromosome 8q24.3 defined by markers D8S1713 and D8S2334. To support this mapping data, seven consanguineous Egyptian families with eight AE patients were genotyped using these markers. Six patients from five families were homozygous in this region. Two point parametric linkage analysis of the Jordanian and Egyptian families gave a maximum LOD score of 4.55 $q=0.001$ at D8S1744. Multipoint analysis using Mapmaker/Homoz resulted in a maximum LOD score of 4.96 in this region. These data place the AE gene within a 3.5 cM interval on chromosome 8q24.3 near the telomere.

Evidence for genetic heterogeneity in hereditary neuralgic amyotrophy (HNA). *G.D.J. Watts¹, K. OBriant¹, T. Borreson², A. Windebank³, P.F. Chance¹.* 1) University of Washington School of Medicine, Box 356320, Seattle, WA 98195; 2) 111 Medical Center Blvd., Suite S750, Marrero, LA 70072; 3) Department of Neurology, Mayo Medical School, 200 1st Street SW, 1501 Guggenheim Building, Rochester, MN 55905.

Hereditary neuralgic amyotrophy (HNA) is a rare autosomal dominant disorder characterized by recurrent episodes of severe arm and shoulder pain with weakness, atrophy and sensory impairment in a brachial plexus distribution. Mild dysmorphic features have also been associated with HNA, including hypotelorism, long nasal bridge and facial asymmetry. Recent studies mapped the HNA locus to chromosome 17q25. We have identified two pedigrees with clinically typical HNA in which markers from chromosome 17q25 do not co-segregate with the disease and that lod scores do not support linkage to chromosome 17q25. These results provide evidence for genetic heterogeneity in HNA and indicate that further studies are needed to map additional gene (or genes) for this disorder.

Homozygosity mapping of quantitative trait loci in complex inbred pedigrees. *M.A. Abney^{1,2}, C. Ober¹, M.S. McPeck^{1,2}*. 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Dept Statistics, Univ Chicago, Chicago, IL.

Founder populations facilitate the search for genes that underlie complex phenotypes because of the relatively small number of independent genomes and recent origins. However, the large, complex genealogies that are characteristic of these populations pose considerable analytical and computational challenges. We have developed homozygosity-based methods to map quantitative trait loci in these populations, and in the Hutterites in particular. We improve on and extend our previous homozygosity-by-descent (HBD) mapping method to detect recessive genes that influence a quantitative trait. By using a hidden Markov method we are able to calculate a likelihood or score statistic at every point in the genome using genotype information at all linked loci and the exact known relationship between the parents of each subject. We assess genome-wide significance by developing permutation-based and simulation approaches. We apply our method to several quantitative traits in the Hutterites.

MERLIN - Multipoint Engine for Rapid Likelihood Inference. *G.R. Abecasis, S.S. Cherney, W.O.C. Cookson, L.R. Cardon.* Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, United Kingdom.

Exact single-point and multi-point inheritance vector calculations are an integral part of haplotyping, linkage analysis and error checking algorithms. Current implementations are restricted to the analysis of a limited number of markers in pedigrees with 20-25 informative meioses.

We describe a fast, memory-efficient engine for traversing the inheritance vector space in pedigrees of moderate size. This tree-based engine automatically detects impossible inheritance vector states and pedigree symmetries, greatly reducing the complexity of the inheritance space, and allowing the analysis of pedigrees with about 40 informative meioses.

The easy-to-use MERLIN computer program uses this engine to perform IBD calculations and state-of-the-art non-parametric linkage analysis and error checking at speeds that can be > 100 times faster than current versions of Genhunter and Allegro. As examples, we show applications of MERLIN to challenging problems, including variance components analysis of quantitative traits in selected samples and enumeration of the most likely inheritance vectors for a pedigree with more than 40 informative meioses.

MERLIN also supports approximate likelihood calculations for the analysis of dense SNP maps with thousands of markers in larger pedigrees (> 25 informative meioses). Fast, flexible programs such as MERLIN will help bring analytical approaches up-to-date with improvements in genotyping technology.

Allele sharing at the IDDM11 and HLA loci jointly predict the risk of IDDM in multiplex families. *E.H. Corder¹, M.A. Woodbury¹, D.K. Madsen¹, L.L. Field²*. 1) Demographic Studies, Duke Univ, Durham, NC; 2) Department of Medical Genetics, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Linkage, and linkage disequilibrium, information for 8 markers spanning the IDDM11 locus and for HLA-DR and -DQ loci were used to predict the risk of IDDM in multiplex families. The sample of 603 sibpairs included those used to map IDDM11 to chromosome 14 (Field et al. 1996). Grade-of-membership (GoM) models were constructed from information on the extent of IBS sharing within pairs. Allelic information was input for markers 3 and 4 thought to bracket IDDM11 and the HLA loci. Seven profiles best represented the data labeled 'I' to 'VII'. IDDM11 sharing for markers 3 and 4 increased over Profiles I to VII: 0, 1, 1, 1, 1 (2), 2(1), 2. Thus Profile I had no, and profile VII complete, IDDM11 sharing. Each profile had a distinct set of shared IDDM11 alleles. HLA sharing was high for profiles I, III (35% HLA-DR 3,4), and VI (95% HLA-DR 3,4). Profile II had no HLA sharing. HLA-related profiles had excess of male pairs. Profile predicted the frequency of doubly affected pairs, ranging from 5% for profile II, i.e. no HLA and average IDDM11 sharing, to 72% for Profile III, i.e. with high HLA sharing and average IDDM11 sharing: 49%, 5%, 72%, 36%, 30%, 69%, 65%. Recurrence risk was expectedly high for Profiles VI (69%, HLA-DR3,4) and VII (65%, complete IDDM11 sharing). In conclusion, inclusion of linkage/linkage disequilibrium information for IDDM11 in the GoM model allowed discrimination between sets of shared HLA alleles which predict diabetes risk.

Assessing Parent-of-Origin Effects in Linkage Analysis of Quantitative Traits. *R.L. Hanson, R.S. Lindsay, S. Kobes, W.C. Knowler.* DAES, NIDDK, Phoenix, AZ.

Some genetic traits are subject to genomic imprinting, but there has been little investigation of methods to account for imprinting in linkage analysis of quantitative traits. The variance components method models the phenotypic variance-covariance matrix (W) among individuals in a pedigree as a function of variance explained by the quantitative trait locus (s_Q^2), residual polygenic variance (s_G^2) and environmental variance (s_E^2). The model is represented by equation 1: ($W = Fs_G^2 + Ps_Q^2 + Is_E^2$, where F is a matrix of kinship coefficients, I is an identity matrix and P is a matrix of the proportion of alleles shared identical by descent by pairs of relatives at the location of interest). In sibships, parent-of-origin effects can be evaluated by partitioning P into components derived from mother (P_M) and father (P_F) and by extending the model to allow s_Q^2 to vary with parent of origin (equation 2: $W = Fs_G^2 + P_Ms_{QM}^2 + P_Fs_{QF}^2 + Is_E^2$).

To examine the statistical properties of this procedure a simulation study was conducted among 956 individuals in a set of 263 sibships who had participated in a genome-wide linkage study and for whom > 1 parent was genotyped. A modification of the procedure of Curtis and Sham (1994) was used to estimate P_M and P_F . For 1000 replicates generated with no linkage between trait and marker loci, type I error rates of a test of $s_{QM}^2 = s_{QF}^2 = 0$ (c^2 with 2 df) were close to nominal values. Similarly, for replicates generated under the model in equation 1, type I error rates for comparison of equations 1 and 2 (c^2 with 1 df) were close to nominal values. When replicates generated under the assumption of an imprinted locus were analyzed under equation 2, median LOD scores for a locus accounting for 10, 30 and 50 % of the variance were 0.4, 4.5 and 12.6 respectively. This compares with 0.3, 3.1 and 7.9 when the same data were analyzed under the model without parent-of-origin effects. These results indicate that modification of the variance components method to incorporate parent-of-origin effects can detect imprinted loci with major effects on quantitative traits without inflation of the type I error rate.

Significance of IBD sharing in genomic mismatch scanning (GMS) experiments. *C. Li¹, R. Spielman², M. Boehnke¹*. 1) Dept. Biostatistics, Univ. Michigan, Ann Arbor, MI; 2) Dept. Genetics, Univ. Pennsylvania, Philadelphia, PA.

Genomic mismatch scanning (GMS) is a high-throughput technology designed to identify region(s) shared identical by descent (IBD) by pairs of individuals. Applying GMS to pairs of distantly-related individuals affected for a genetic disease is a potentially powerful and efficient approach to identify regions that harbor disease-predisposing genes, since it in principle allows the scoring of all intermediate meioses simultaneously. Cheung et al. (1998) presented a proof-of-principle analysis of GMS on Ashkenazi Jews with congenital hyperinsulinism, an autosomal recessive disease whose gene SUR1 maps to chromosome 11p15.1. They observed a maximum of seven matches along chromosome 11 in eight not known to be related pairs. Based on a conservative Bonferroni adjustment, they calculated a p-value of .0003 for this result. For continuous IBD observations, Feingold (1993) derived a method to derive approximate p-values for this sort of experiment as a function of degree of relationship of the relative pairs. Applying this method, we calculated the p-value for the Cheung et al. data. Even assuming the eight pairs all are as closely related as second cousins, the probability of observing seven or more IBD matches along chromosome 11 is .000002, much smaller than the p-value calculated by Cheung et al. This p-value is even smaller for more distant relationships, which probably are more likely for these data. Observed IBD data from GMS actually are discrete rather than continuous. Thus, the actual p-values for these data are still smaller than the ones we calculated, which assume continuous IBD data. We will address the impact of this issue of marker density. We also will address by computer simulation the goodness of the Feingold approximation for distant relative pairs, and estimate statistical power under several disease models. Finally, we will consider the impact on significance and power of analyzing data on dependent relative pairs.

PROFILER: a program to compute cumulative probability profiles with applications to pedigree analysis. *J.R. O'Connell*. Department of Human Genetics, University of Pittsburgh, PA.

PROFILER is a software program that uses efficient recursive genotype elimination and rapid multipoint likelihood algorithms to compute the probability of joint genotype vectors in pedigree data. In particular, given a pedigree P , P' a subset of n individuals of P , a set of markers M , M' a subset of k markers of M , let G be the set of all vectors (G_1, G_2, \dots, G_n) , where G_j is a multilocus genotype of individual j consisting of markers from M' , that are consistent with the pedigree data. First, for each genotype vector g in G , PROFILER computes $p_v = \text{prob}(v|P, M)$. PROFILER then sorts these probabilities in decreasing order, and computes the cumulative probability for each vector. The cumulative probability of v is p_v plus p_w , where w is any vector appearing in the sorted list before v . These probabilities define the cumulative probability profile of P' over M' with respect to P and M .

These profiles offer a powerful tool to perform a variety of important pedigree analyses including haplotyping, computing IBD probabilities, identifying genotyping errors, risk calculations and approximating the likelihood. For example, to compute the IBD probabilities for a pair of individuals I_1 and I_2 at a position x on a framework map of markers, let $P' = \{I_1, I_2\}$, M' be a fully informative dummy marker defined by assigning each founder a distinct pair of alleles and placed at x , and M be M' plus some flanking markers. Compute the profile for $\{I_1, I_2\}$ with respect to the dummy locus and then sum up the probabilities of each vector sharing 0, 1 or 2 alleles, respectively. These IBD probabilities, which are important for non-parametric linkage analysis and variance components methods, can be computed for general pedigrees using PROFILER.

The power of PROFILER comes from being able to use a larger set of markers M when computing probabilities over M' . These additional markers can give a much more accurate profile of the data over M' relative to the framework map than those of M' alone. Detailed examples of the performance of PROFILER applied to the analyses listed above are presented.

Model-dependent linkage analysis and Type I error rate under random ascertainment. *D.M. Mandal¹, A.J.M. Sorant², A.F. Wilson², J.E. Bailey-Wilson².* 1) Louisiana State University Health Sciences Center, New Orleans, LA; 2) National Human Genome Research Institute, NIH, Baltimore, MD.

Model-dependent lod-score linkage analysis is expected to be robust with respect to Type I error rate when ascertainment is random. Previous studies of simulated data for a quantitative trait and a highly polymorphic marker locus showed that when the generating ("true") model for the trait was assumed in the analysis, the power of lod-score linkage analysis was increased when the marker allele frequencies were misspecified and parental data were missing. Model parameter estimates from segregation analysis of large simulated sample data sets were extremely close to the generating models; thus, errors in the simulation were ruled out as the cause of this unexpected result. This prompted the investigation of Type I error rates. When marker allele frequencies were severely misspecified and parental data were missing, a large increase in Type I error rate was observed, in some situations by as much as 10 times the nominal level. In an attempt to remedy this problem, additional simulations (G.A.S.P.) were conducted in which the lod-score linkage analyses (LODLINK, S.A.G.E. v. 3.1) were performed using a model estimated from each sample of the simulated data, using segregation analysis (REGCHUNT), rather than simply using the generating model for analysis. In this situation, the Type I error rate was not inflated above the expected nominal level. These results suggest that the previously observed inflated Type I error rates (when marker allele frequencies were misspecified and the generating model for the trait was assumed) were probably due to discrepancies between the generating model and the trait model that best fit each sample of the data. Therefore, in order to avoid spurious inferences of linkage, both trait model parameters and marker allele frequencies should be estimated from the sample data when performing linkage analysis of randomly ascertained quantitative traits.

A genome-wide stepwise linear discriminant analysis approach to the genetic analysis of IDDM. *D.R. Nyholt.*
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Linear discriminant analysis (DA) provides linear functions of independent variables that "best" separate cases into two or more predefined groups (the dependent variable). The first discriminant function maximizes the differences between the values of the dependent variable; that is, where the group means are as different as possible. The second function is then computed which is orthogonal (uncorrelated) to the first; i.e., the second discriminant function maximizes the differences between values of the dependent variable while controlling for the first function (variable). A third function is then computed uncorrelated with the first two, and so on, for as many functions as possible.

In terms of genome-wide linkage scans, marker loci may be thought of as independent variables and affection status the dependent variable. For example, in an affected sibpair study, "case" data would be directly calculated by examining IBD allele sharing between affected sibpairs, while "control" data could be obtained after generating (by computer simulation) marker data into the family members and analogously examining IBD sharing. This is done independent of disease inheritance, but based on the known laws for marker inheritance. One would then compare observed versus generated family data. Although genome-wide linkage scans utilize many (200-400) markers, they have a relatively small number of observations. Therefore, a stepwise approach is necessary to select the variables (loci), which contribute most to the separation between groups. Stepwise procedures select the most correlated independent variable first, remove the variance in the dependent, and then select the second independent variable which most correlates with the remaining variance in the dependent. This process continues until selection of an additional variable does not increase the inclusion criteria (e.g. F-statistic) by a significant amount (usually significance = 0.05).

I propose the use of stepwise DA in an initial exploratory phase of genome-wide linkage data, and demonstrate its usefulness by application to the John Todd IDDM data.

The posterior probability of linkage (PPL) incorporating prior genomic information is efficient for detection of linkage and estimation of male/female recombination rates. *V.J. Vieland, E.A. Ludington, K. Wang, J. Huang.* Univ Iowa, Iowa City, IA.

By utilizing prior information on the genetic map, including intermarker distances and known differences in sex-specific recombination fractions q_M and q_F , we are able to calculate the PPL, or the probability of linkage given the data. We have argued elsewhere that the PPL has advantages over standard methods, particularly when mapping complex-disease genes based on multiple heterogeneous data sets; and we have shown that the PPL converges to its true value (1 under linkage; 0 otherwise) as N goes to ∞ even under model misspecification. Here we begin to investigate the rate of convergence based on simulated 2-3 generation pedigrees with 32 affecteds, under a range of simple and complex generating models, various prior distributions for (q_M, q_F) , and assuming a marker map of either 5 or 20 cM average spacing. The table below shows the mean and s.d. of the PPL as a function of sample size N , for a dominant disease with low penetrance (g.f.=.10, pen=20%), a 5 cM marker map, and average genetic distances between the trait and marker of $q_M=.003$, $q_F=.021$ (7:1 female:male ratio).

	N=20	N=40	N=60	N=80	N=100
PPL	0.60	0.89	0.98	0.99	1.00
s.d.	0.37	0.26	0.11	0.08	0.00

When there is no linkage, the mean PPL=0.01 (s.d. 0.02) for $N=20$ and by $N=100$ the mean PPL=0.00 (s.d. 0.00). Thus the PPL converges efficiently towards 1 (under linkage) and 0 (under no linkage) even for moderate sample sizes. In addition, by $N=20$ families, estimation of (q_M, q_F) is extremely accurate, with average bias of only 0.00 (q_M) and 0.01 (q_F). This shows that the PPL can yield clear evidence for or against linkage and excellent estimation of (q_M, q_F) in realistic sample sizes.

Transmission/disequilibrium tests using multiple tightly linked markers. *H. Zhao¹, S. Zhang¹, K.R. Merikangas¹, D.B. Wildenauer², F. Sun³, K.K. Kidd⁴.* 1) Department of Epidemiology & Public Health, Yale University School of Medicine, New Haven, CT; 2) Department of Psychiatry, University of Bonn, Germany; 3) Department of Mathematics, University of Southern California, Los Angeles, CA; 4) Department of Genetics, Yale University School of Medicine, New Haven, CT.

Transmission/disequilibrium tests have attracted much attention in genetic studies of complex traits because of the possibility that they may have greater power than other linkage methods to detect genes having small to moderate effects, and they are robust against population stratification. Highly polymorphic markers have recently become available throughout the genome and many such markers can be studied within short physical distances. Studies using multiple tightly linked markers are more informative than those using single markers. However, such information has not been fully utilized by existing statistical methods, resulting in possibly substantial loss of information in identifying genes underlying complex traits. We will discuss several novel statistical methods for analyzing multiple tightly linked markers. Strengths and limitations of existing methods for analyzing multiple markers are reviewed. Simulation results suggest that our proposed methods are more powerful than existing methods. Finally, we present the results of application of the proposed methods to empirical data from a genetic study of the dopamine D2 receptor and alcoholism. Among the 77 family trios studied, there were 55 German families and 22 Hungarian families. Three polymorphisms spanning 30kb within the DRD2 locus were genotyped. Using the proposed multilocus methods, we found that the general transmission patterns were similar among the two populations, although it was more extreme for the Hungarian families. Supported in part by USPHS grants GM59507, GM57672, and HD36834.

Genetic heterogeneity and the analysis of complex traits. *C.T. Falk.* Population Genetics, New York Blood Center, New York, NY.

Linkage analysis of complex genetic traits poses a number of challenging problems. One class of complex traits results from genetic heterogeneity, where two or more independent, unlinked loci lead to a single disease phenotype. Since the ultimate goal is to find and clone the gene(s) responsible for a disease, we need methods to reliably identify a small chromosomal region harboring a single disease locus. The first step is linkage analysis of families with the disease and, once close linkage to previously mapped marker loci is found, the region can be narrowed by molecular methods. The presence of a second disease locus leading to an unlinked but indistinguishable form of the disease provides a level of "noise" in the data that makes this difficult. It has been shown that, in the presence of genetic heterogeneity, estimates of the recombination fraction, based on linkage analysis, will be grossly over estimated and regions harboring a disease locus may actually be excluded by the linkage results. Some methods of analysis allow for such heterogeneity, assuming, e.g., that some families are linked to a set of markers and the remaining families are not. These methods jointly estimate the recombination fraction and the proportion of linked families and produce a more reliable estimate of the distance between a marker locus and a putative disease locus. They do not, however, identify which families have the linked form of the disease. Thus when the search requires molecular methods, it is not possible to determine reliably which families to study further. We propose a strategy for the analysis of heterogeneous genetic traits that combines 2-point linkage analysis and heterogeneity analysis with a series of steps to select out a subset of families having a high probability of being of the desired, linked form of the disease. We illustrate the method with simulated family data and show the effect of changes in several variables on the success of the method. In general, the method improves as the number of available linked markers increases and as the distance between the markers decreases. Under reasonable conditions, 70-80% of the families of the linked form can be identified, with a false positive rate of 5% or less. Support: GM29177.

Incorporating clinical data into analysis of susceptibility loci suggests *CAPB* linkage among prostate cancer families with a high-grade case. *E.L. Goode*¹, *J.L. Stanford*², *M. Gibbs*², *M. Janer*³, *M. Peters*², *S. Kolb*², *M.D.*

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Hereditary prostate cancer is a heterogeneous complex disease with at least 4 susceptibility loci mapped to date. We assessed linkage to multiple markers at *CAPB* (1p36), *HPC1* (1q24-25), *PCaP* (1q42.2-43), and *HPCX* (Xq27-28) in 149 families with 3 or more living affected men using clinical data from the medical records of 505 affected men. LINKAGE, HOMOG, and GENEHUNTER were used to examine linkage and heterogeneity. Overall, maximum 2-point lod scores were: 0.86 ($q=0.18$) at *CAPB* (D1S407), 0.43 ($q=0.24$) at *HPC1* (D1S1660), 0.57 ($q=0.26$) at *PCaP* (D1S2785), and 0.16 ($q=0.34$) at *HPCX* (DXS984). When analyzing a complex disease, considering clinical characteristics may identify homogeneous subgroups and thus improve power to detect linkage. Therefore, we used distributions of prostate cancer grade and stage and median age at diagnosis to stratify families, in addition to race and lod scores at other loci. A maximum NPL score of 1.83 ($p=0.04$) was seen at *CAPB* (D1S407) for 37 white families with at least 1 high-grade cancer; this rose to 2.34 ($p=0.01$) when families with evidence for other linkage were removed. These results suggest that *CAPB* may be involved with high-grade prostate cancer and that, as expected, this involvement is more apparent when genetic homogeneity is increased. At *HPC1*, the most suggestive results were for 42 white families with median age > 70 years, with a peak NPL of 1.59 ($p=0.06$, D1S1660) and positive lods and NPLs at all markers. In the *PCaP* region, a peak NPL of 1.03 ($p=0.15$) was seen at D1S1609 for 26 white families with moderately differentiated tumors and no evidence for other linkage. A 2-point maxlod of 1.14 ($q=0$) was seen at *HPCX* (DXS1193) for 33 white families with median age 60-64 years. Considering clinical data as well as evidence for linkage to other loci can improve genetic homogeneity and may prove useful in understanding multiple loci responsible for hereditary prostate cancer.

The transforming growth factor-b1 locus (TGFB1) on ch.19q13 and clinical phenotypes in familial multiple sclerosis (MS). *A.J. Green¹, L.F. Barcellos¹, J.B. Rimmeler², S. Caillier¹, P. Bucher¹, R.R. Lincoln¹, M.E. Garcia³, J.L. Haines³, M.A. Pericak-Vance², S.L. Hauser¹, J.R. Oksenberg¹.* 1) Department of Neurology, University of California, San Francisco; 2) Center for Human Genetics, Duke University; 3) Program in Human Genetics, Vanderbilt University.

Full genome screenings in MS families have identified multiple susceptibility regions supporting a polygenic model for MS. Evidence for linkage was observed at ch.19q13 suggesting the presence of an MS gene of modest effect. Several candidate genes are encoded here, including TGFB1. Transforming growth factor-b1 is a multifunctional cytokine with important immunomodulatory properties. Both patient and animal model studies provide strong evidence for the role of TGF-b1 in MS pathogenesis. Several polymorphisms have been identified within TGFB1; at least 2 variants result in functional differences associated with variation in production levels, both in vitro and in vivo. We performed a comprehensive evaluation of the ch.19q13 region, including 5 TGFB1 polymorphisms and 4 flanking markers (D19S421, CEA, D19S908, IL-11), in 141 stringently ascertained MS families (412 affecteds, 246 ASP) using linkage and association tests. Patients and families were stratified by presence or absence of HLA-DR2 and clinical MS phenotypes for analyses of single loci and extended TGFB1 haplotypes. Modest linkage was observed for CEA (lod score=1.1, $q=0.30$), SimIBD ($p<10^{-4}$) located 0.4 cM from TGFB1. Significant results were not present for other ch.19q13 loci or haplotypes in families considered together or when stratified by HLA-DR2. When distinct clinical phenotypes were utilized for analyses, significant results were observed in families sharing common first symptoms. Families in which optic nerve/spinal cord involvement was not present at first onset ($n=29$) demonstrated modest linkage (lod score=1.3, $q=0.00$), ASPEX (MLS=0.74), SimIBD ($p=0.013$) and association ($p=0.01$) with the TGFB1 SNP at codon 10. These findings further support a susceptibility locus in 19q13 and suggest that TGFB1 or a nearby locus may influence disease susceptibility, and emphasize the importance of considering clinical information in efforts to identify MS genes.

Progress toward Gene Identification for Alcohol-Related Phenotypes. *M.A. Ehringer¹, J. Thompson¹, O. Conroy¹, Y. Xu¹, F. Yang¹, J. Canniff¹, M. Beeson², L. Gordon², B. Bennett², D. Goldman³, M. Schuckit⁴, T.E. Johnson², J.M. Sikela¹.* 1) Dept Pharmacology, Univ Colorado Health Sci Ctr, Denver, CO; 2) Inst Behavioral Genetics, Univ Colorado, Boulder, CO; 3) National Inst Alcoholism and Alcohol Abuse, NIH, Rockville, MD; 4) Dept Psychiatry, Univ California San Diego, San Diego, CA.

A low intensity of reaction to alcohol has been shown to be one of the best predictors for future alcohol abuse or dependence. ISS (Inbred short-sleep) and ILS (Inbred long-sleep) mice are considered a model system for the study of initial sensitivity to alcohol, as they were selectively bred for a difference in the loss of righting reflex upon ethanol exposure. Four QTLs in the mouse genome have been previously identified; each contains one or more genes that contribute to alcohol sensitivity. By searching the current human and mouse genome maps, we have identified 461 genes located within the QTLs and ranked them based on their likelihood to be involved in an alcohol-related phenotype. Using a high-throughput sequencing approach, we have comparatively surveyed the coding regions of 86 genes between ISS and ILS mice. This corresponds to over 1.5 million nucleotides of sequencing reads, representing approximately 38,000 amino acids. Novel amino acid differences between ISS and ILS mice have been detected in eight genes. The location of these genes within their respective QTLs is being confirmed using heterozygous recombinant congenic animals, which were developed to facilitate narrowing of the intervals. To date, three genes were found not to localize to QTL regions and three others are currently being remapped using this method. The remaining two genes, both of which are members of the zinc finger family of proteins, have been localized to the narrowed intervals. We have begun sequencing one of these zinc finger proteins in a human population in an effort to identify novel coding region SNPs that might be related to alcohol use disorders. Such sequence differences could be useful for further association or linkage studies in humans.

A genome-wide linkage scan reveals evidence for regions that influence variation in nine plasma lipid and apolipoprotein levels. *K.L.E. Klos¹, S.L. Kardina¹, R.E. Ferrell², S.T. Turner³, E. Boerwinkle⁴, C.F. Sing¹*. 1) University of Michigan, Ann Arbor, MI; 2) University of Pittsburgh, Pittsburgh, PA; 3) Mayo Clinic, Rochester, MN; 4) University of Texas Health Science Center, Houston, TX.

We carried out a linkage study to identify new regions of the genome that have genetic loci that influence quantitative variation in measures of risk of coronary artery disease (CAD). We utilized 373 marker loci measured on 1484 individuals distributed among 232 three generation pedigrees sampled from the Rochester, MN population without regard to their health status. LOD scores and estimates of additive genetic variance associated with map locations were obtained using the variance-component method of linkage analysis. No evidence for linkage when considering age as the trait of interest served as a negative control while a highly significant LOD score (4.203) associated with the apolipoprotein (Apo) E gene region when considering plasma Apo E level served as a positive control. No other LOD score exceeded 3.0. There were 6 regions that gave a LOD score of greater than 2.0 in 5 traits. Thirteen additional regions had LOD scores ³ 1.4 involving 8 traits. Contrasting results from adjusted and unadjusted data, transformed and untransformed traits and analyses based on different generations suggest that the variance-component approach to linkage is robust for regions associated with single loci determining heritabilities greater than 0.50 while identification of genes with smaller effects depends on the definition of the trait of interest and the context defined by the sample being analyzed. This research was supported by NIH grants HL39107, HL51021 and HD32197.

Exclusion of *CACNA1A* and *KCNN1* as candidate genes for migraine in three 19p13-linked Finnish families. *M. Kaunisto*^{1,2}, *E. Hämäläinen*², *M. Kallela*³, *H. Harno*³, *P. Marttila*⁴, *I. Hovatta*¹, *A. Orpana*², *L. Peltonen*⁴, *M. Färkkilä*³, *A. Palotie*⁴, *M. Wessman*^{1,2}. 1) Department of Biosciences, University of Helsinki, Helsinki, Finland; 2) Laboratory Department of Helsinki University Central Hospital, Helsinki, Finland; 3) Department of Neurology, University of Helsinki, Helsinki, Finland; 4) Departments of Pathology and Human Genetics, UCLA School of Medicine, LA, USA.

Migraine is a common neurological disorder caused by a combination of genetic and environmental factors. Familial hemiplegic migraine (FHM) is a rare autosomal dominant form of migraine with aura characterized by transient hemiparesis or hemiplegia during the migraine attack. Half of the FHM families are known to be linked to 19p13 where the brain specific P/Q-type calcium channel α_{1A} -subunit gene, *CACNA1A*, is located. Several different missense mutations in this gene have been shown to cause FHM. The same locus has also been suggested to be associated with the more common forms of migraine. For some of the FHM families linked to 19p13 studies have failed to identify mutations in *CACNA1A*. Therefore, it is possible that mutations in some other gene located within this region could cause FHM or more common forms of migraine.

We have studied three 19p13-linked migraine families, two of them suffering from FHM and one from migraine without aura. The diagnoses were made according to the IHS criteria and were based on either a clinical evaluation or a validated questionnaire. All 47 exons and the recently identified exon 37B of *CACNA1A* were analyzed in these families by sequencing. Four new and ten previously described polymorphisms were observed but no potential disease causing mutations were found. We also analyzed the human SK1 gene *KCNN1* that was recently mapped to 19p13. Since SK channels are small-conductance, calcium-activated potassium channels and regulate neuronal excitability the corresponding genes are good candidates for migraine. We sequenced all 11 exons of the *KCNN1* gene from our three families. One polymorphism was detected but no potential mutations were found.

Genetic studies in familial Ankylosing Spondylitis. *J. Luo^{1,2}, J.M. Akey^{1,2}, J. Bruckel^{1,3}, R. Inman^{1,4}, M. Weisman¹, M.A. Khan¹, R. Schumacher^{1,5}, M. Mahowald¹, D.T. Yu¹, L. Jin^{1,2}, J.D. Reveille^{1,2}.* 1) The North American Spondylitis Consortium; 2) Human Genetics, University of Texas, Houston; 3) Sherman Oaks, CA, 91403; 4) Toronto ON, M5T2S8; 5) Philadelphia PA, 19104.

Ankylosing Spondylitis (AS) is a common complex inflammatory disease of the spine and back, which affects between 0.5-2% of the general population. While 90-95% of AS patients carry the HLAB-27 allele, other genetic factors also contribute to disease susceptibility. Previous studies have implicated regions on chromosomes 6p, 2q, and 16q in predisposing to (AS). In order to confirm and extend these findings an independent cohort of multiplex AS families and affected sib pairs were collected from the Spondylitis Association of America and nine University-based practices in the United States and Canada. Only those families who met stringent phenotyping requirements (modified New York criteria for AS and available radiographs) were included. Furthermore, all of the affected individuals in this study were HLA-B27 positive. In total, we genotyped 53 sib pairs from 47 families for microsatellite markers on chromosome 6p, 2q, and the telomeric end of 16q. Moreover, we conducted extensive HLA-B27 and HLA class II sub-typing (DRB1, DQA1, and DQB1) in all family members. Linkage analysis was performed with Genehunter using the NPL statistic. All of the markers around the major histocompatibility complex (MHC) showed linkage ($p \leq 0.01$), although whether this reflected the sole effect of HLA-B27 or another MHC gene remains to be determined. Marker D2S340, located near the IL1 gene complex, showed nominal evidence for linkage ($p=0.07$). No evidence for linkage was found for markers at the telomeric end of chromosome 16q ($p=0.7-0.9$). These data suggest a role for both MHC and non-MHC genes in mediating susceptibility to AS. Further studies with larger numbers of sib pairs and additional markers are in progress. More generally, this study demonstrates the value of strict phenotyping in genetic studies of complex disease as significant results were obtained with a rather small sample size.

Linkage studies to asthma and atopy phenotypes of chromosomes 7, 12, and 19, in the Italian population. G. Malerba¹, S. Venanzi¹, R. Galavotti¹, C. Migliaccio¹, M.C. Lauciello¹, E. Trabetti¹, G. Zanoni², L. Pescollderungg³, L.C. Martinati¹, A.L. Boner¹, P.F. Pignatti¹. 1) Mother-Child & Biol, Genetics, Univ Verona, Verona, Italy; 2) Dpt Pathology, Univ Verona, Verona, Italy; 3) Division Pediatrics, Hospital Bolzano, Italy.

Chromosomes 7, 12, and 19, were investigated in 117 Italian families with allergic asthmatic children sib-pairs. Chromosome 7: showed suggestive non parametric lod (NPL) scores for clinical asthma (NPL=2.62; p=0.0045), elevated IgE (NPL=2.61; p=0.005), and atopy (NPL=2.61; p=0.005), with marker D7S2846. Chromosome 12: we previously described a suggestive linkage of marker D12S390 with clinical asthma. Further analysis with 5 additional markers mapping in the region confirmed the result (D12S270, NPL=3.12; p=0.0006). Chromosome 19: we have reported a suggestive linkage to marker D19S601 for atopy, elevated IgE and skin prick test reactivity. Further analysis with 8 additional markers indicated a reduction in NPL value (D19S571, NPL=1.69; p=0.046). A simulation study using 17 markers (from D19S433 to D19S210, 47cM) indicated no suggestive linkage (p=0.06). Our study shows that it may be useful to perform a second analysis with other DNA markers in regions showing suggestive linkage. Genes on chromosome 12, and possibly, after confirmation, on chromosome 7, could be involved in the susceptibility to allergic asthma. Acknowledgments: Supported by Telethon Italy and M.U.R.S.T. Italy.

Mapping the genetic component of SLE: linkage and linkage disequilibrium mapping in an isolated population.

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Systemic lupus erythematosus (SLE) is a prototype of an autoimmune disease affecting multiple organ system: joints, skin, kidneys and central nervous system. Our aim is to map and identify susceptibility genes for SLE in the Finnish population, with a prevalence of 28/100 000. Through the years 1995-1998 we identified approximately 1200 SLE patients from all over the Finland representing approximately 80-85% of all Finnish SLE patients requiring hospital treatment. The patients were contacted and their medical records were used to verify their diagnosis (at least 4/11 ARA criteria fulfilled). Over 250 families with one or more affected pedigree members have been ascertained. In 53 families there were two or more affected pedigree members in a family. Forty-six families had two affected members and 7 families had three affected members. There were three pairs of monozygotic female twins and one pair of dizygotic twins of the opposite sex concordant for SLE. Eleven (9.7%) of the 113 familial cases of SLE were male. No differences were found in the clinical presentation of SLE between familial and sporadic cases (sex, age at onset, major clinical manifestations and common laboratory tests). Among first degree relatives, time intervals between the ages and dates at onset of SLE were similar (median 5.9 years and 7.0 years, correspondingly). In twins, the intervals were short and almost equal (4.0 years and 4.0 years). In a subset of these families (35 families) we have conducted a genome wide scan using of 400 polymorphic microsatellite markers evenly spaced throughout the genome. The data-set is being analyzed using both non-parametric and parametric linkage analysis. Our preliminary simulations of the data-set indicate that we have a power of 90% to detect a locus present in 80% of the families using the non-parametric analysis approach.

Genome scan for asthma in a young founder population. *T. Laitinen*¹, *M.J. Daly*², *J.D. Rioux*², *E.S. Lander*², *T.J. Hudson*², *J. Kere*¹. 1) Dept Medical Genetics, Univ Helsinki, Helsinki, Finland; 2) Center For Genome Research, Whitehead Institute for Biomedical Research, MIT, MA.

Asthma and other atopic disorders are common and genetically a heterogeneous group of diseases. We carried out a genome-wide scan for asthma in a young founder subpopulation from central eastern Finland. Present population of 100,000 represents descendants of a few hundred founders after 20-25 generations (long and narrow genetic bottleneck and rapid expansion). Two regions of suggestive linkage were initially identified and studied further with higher density mapping. The significance of linkage increased sharply in one of the two loci. This region was then subsequently analyzed for linkage using the high density map of genetic markers and altogether three asthma related phenotypes: asthma, high serum IgE, and the presence of both. We chose to use a clinically meaningful dichotomy for serum IgE values (cut-off point at 100 kU/L) to be able to combine phenotypes for linkage analysis. Using non-parametric multipoint linkage analysis (GENEHUNTER 2.0), the best NPL scores for respective phenotypes were 2.8 (P=0.003, information content 0.71), 3.9 (P=0.0001, information content 0.76), and 3.1 (P=0.002, information content 0.77). The genome wide significance of linkage was evaluated using simulations in which the artificial data set matched with our marker density, informativeness, and exact pedigree structure. In simulations, which conservatively use a high density marker map across the entire genome, an NPL score exceeding 3.9 was seen 28 times in 1000 genome scans, giving an empirical genome-wide p-value of 0.028. This p-value is below the P=0.05 threshold declaring genome-wide significance (i.e. the score one would expect once in 20 genome scans by chance). Based on simulated data sets, it has been argued that the choice of a study population can be the most critical issue when gene mapping studies for complex traits are designed. Our study supports the hypothesis that genetic isolates may be advantageous for identifying major loci for complex traits.

Mapping a Schizophrenia Susceptibility Locus on Chr13q32: Searching for Candidate Genes. *J.A. McDonough¹, K.V. Chowdari², S. Shaikh¹, G.C. Nickel¹, Z. Feng², J. Wood², V.L. Nimgaonkar², A. Chakravarti¹.* 1) Department of Genetics, Case Western Reserve University, Cleveland, OH 44106; 2) University of Pittsburgh School of Medicine, Western Psychiatric Institute and Clinic, Pittsburgh, PA 15213.

Previous linkage studies have consistently shown that Chr13q32 may harbor susceptibility genes for schizophrenia. We have created a physical map of this region by screening the Roswell Park RPCI-11 human BAC library with Chr13 markers. We identified 93 BAC clones covering the Chr13q32 region and have assembled a contig covering ~5Mb surrounding the marker D13S174. Using this map, we have genotyped 7 microsatellite markers spanning 20cM with inter-marker distances up to 9cM. Linkage analysis among 40 affected sib pair families from the NIMH schizophrenia collection yielded a maximum LOD score of 1.38 and a NPL score of 2.92 ($p < 0.0016$) at D13S174, under a recessive model. Suggestive evidence for linkage disequilibrium was also obtained using TDT analysis of 159 other simplex families. We are searching for brain expressed candidate genes in this region using unfinished BAC sequences generated by the Sanger Centre. We have mapped sequences from 10 genes to the contig and have begun sequencing through exons and adjacent introns in order to identify single nucleotide polymorphisms (SNPs). A potential candidate gene, tripeptidyl peptidase II (TPP2), maps to ~170-340kb from D13S174. The TPP2 gene is a serine peptidase which degrades the neuropeptide, cholecystokinin (CCK). We have determined the genomic organization of the TPP2 gene from direct sequencing from BACs and from unfinished sequence. We have identified three SNPs, two in introns and one in the 3'UTR and have begun genotyping these SNPs to test for association to schizophrenia in our families.

PSEUDOMARKER: Computer software for linkage and linkage disequilibrium analysis of complex traits. T.

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Göring and Terwilliger (2000) described a set of statistical tests for "model-free" linkage and/or linkage disequilibrium on mixtures of singletons, triads, sibpairs and larger pedigrees, which is rather difficult to apply in practice using the conventional software packages. We have developed a series of programs, PSEUDOMARKER, for application of these test statistics in a fully automated manner. First, we had to convert disease phenotypes into pseudomarker genotypes according to the algorithm described by Göring and Terwilliger (2000). Second, the difficult issue of maximizing the likelihoods as required to perform the requisite statistical tests needed to be addressed as well. One needs to maximize the likelihood over the marker locus allele frequencies, under the assumption of no linkage, $L_1 = \max_{p_i} L(p_i, q=0.5)$, the likelihood maximized over both allele frequencies and the recombination fraction jointly, $L_2 = \max_{p_i, q} L(p_i, q)$ the likelihood maximized over marker-trait haplotype frequencies under the assumption of no linkage, $L_3 = \max_{q_{i,j}} L(q_{i,j}, q=0.5)$, and the likelihood maximized over both linkage and haplotype frequencies jointly, $L_4 = \max_{q_{i,j}, q} L(q_{i,j}, q)$. The lod score for linkage, ignoring any LD would be $\log_{10}(L_2/L_1)$, with a lod score statistic to test LD, given linkage, is $\log_{10}(L_4/L_2)$. A test for linkage given prior knowledge of LD could be conducted as $\log_{10}(L_4/L_3)$. The PSEUDOMARKER is comprised of two parts. One module for nuclear pedigrees, triads, and singletons alone, exists as a stand-alone program written in C, which can compute the four likelihoods described above for two-point analysis of either pseudomarker-based or model-based analyses. The second module is a series of shell scripts written in a mixture of C and Perl, which calls the ILINK program of the LINKAGE package serially to maximize the likelihoods on any available data structures. Software is available from <http://www.genetics.ucla.edu/software/pseudomarker>.

Two Candidate Regions for Schizophrenia and Bipolar Disorder on Chromosome 18: Linkage and Association Studies in Ashkenazi Jewish Family Samples. *A.E. Pulver¹, V.K. Lasseter¹, P.S. Wolyniec¹, K.L. Swartz¹, G.*

Nestadt¹, M.H. Thornquist¹, K-Y. Liang², J.W. Barlow³, C.B. Hunter¹, M.S. Carswell¹, H.L. Peay¹, K. Ewen³, J.A. McGrath¹. 1) Dept. Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Dept. Biostatistics, Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD; 3) Australian Genome Research Facility, Victoria, Australia.

Both schizophrenia and bipolar disorder are common psychiatric diseases with likely complex modes of inheritance. Linkage studies in bipolar disorder have implicated two regions on chromosome 18: 18q21-q22 (Stine et al., 1995; McMahon et al., 1997) and 18p11 (Berrettini et al., 1994; Stine et al., 1995). Linkage and association studies in a sample of 59 multiplex schizophrenia pedigrees from Germany and Israel have also suggested that the 18p11 region may harbor a schizophrenia susceptibility locus (Schwab et al., 1998). Some overlap in susceptibility genes for the two disorders is hypothesized. Disequilibrium studies in genetically isolated populations may be more likely to succeed in narrowing susceptibility intervals due to the likelihood of founder effects. Linkage disequilibrium studies and/or association studies in 223 Ashkenazi schizophrenia triads and 152 Ashkenazi bipolar triads use genotypings from a dozen microsatellites in each of the two regions. We selected markers covering approximately 21 cM in each region (D18S59-D18S45 in 18p and D18S64-D18S461 in 18q) with an average intermarker distance of 1.9 cM. The data are examined for associations of disease status with allele frequencies, genotype frequencies, for deviation from expected Hardy-Weinberg Equilibrium (HWE), and for distortions in expected allelic transmission rates using transmission disequilibrium testing (TDT) methods. Berrettini WH, et al. (1997) Arch Gen Psychiatry 54:27-35; Berrettini WH, et al. (1994) Proc Natl Acad Sci U S A 91:5918-21; McMahon FJ, et al. (1997) Am J Hum Genet 61:1397-404; Schwab SG, et al. (1998) Am J Hum Genet 63:1139-52; Stine OC, et al. (1995) Am J Hum Genet 57:1384-94.

The search for cancer modifier genes using single nucleotide polymorphisms at human chromosome 20q13.2.

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The majority of sporadic cancer is due to environmental factors and inherited cancer modifier genes. In humans, these low penetrance genes are difficult to identify using traditional methods. However, mouse models using inbred mouse strains such as *Mus spretus*, which is relatively cancer resistant, and *Mus musculus*, which is relatively cancer susceptible, can be used to map these genes. Once a locus is identified, mouse models can be used to further refine the locus and identify candidate genes. Alternatively, if the orthologous locus in humans is well characterized, polymorphisms within candidate genes can be used for association studies. We mapped several mouse cancer modifier loci using this approach. One locus, Skts13 on distal chromosome 2, shows strong linkage for skin tumor susceptibility with a p-value of 1.9×10^{-9} using 261 mice (Wilcoxon rank sum test). Approximately 18% of mouse skin tumors from backcross mice and 63% of lymphomas from radiation exposed F1 mice show imbalance of distal 2. This provides additional evidence for a tumor susceptibility locus mapping to mouse distal 2. This locus was also intriguing to us because the syntenic region in humans, 20q13.2, is amplified in a variety of tumor types and contains at least 4 potential candidate genes. To identify polymorphisms, including single nucleotide polymorphisms (SNPs), that may be used for association studies to identify the genes of interest at this locus, we sequenced the coding region and intron/exon boundaries of 4 candidate genes, *AURORA2*, *ZNF217*, *PIC1L*, and *CYP24* in 13 to 16 individuals. 31 SNPs were identified which were used to construct haplotypes for these genes. Haplotypes are currently being refined using larger numbers of cancer cases and controls. To date, regions of linkage disequilibrium in these genes are small and appear to be less than 10kb. This fits with some estimates of linkage disequilibrium made for other loci. To identify which genes at 20q13.2 are involved in tumor susceptibility, association studies will be conducted using select SNPs specific for haplotypes seen more frequently in cancer cases or those that may confer a functional change in the protein.

Serotonin receptor 2A T102C and severe myopia. *E.C. Tan, P.L. Chia, R.Y.Y. Yong, H.M. Wu, E.P.H. Yap.* Defence Medical Research Institute, Defence Science & Technology Agency, Singapore.

Myopia affects only about 25% of Caucasians but the prevalence rate is two to three times higher in Asian populations. For young males in Singapore, the prevalence of myopia exceeds 80%. While environmental factors such as near work and educational attainment are important, family history and ethnic background are also significant risk factors suggesting genetic predisposition in the development and progression of severe myopia. In our ongoing study of susceptibility genes for myopia, retinally-expressed genes are examined as functional candidates. The presence of 5-HT_{2A} receptors have been demonstrated in both rabbit and goldfish retinal membrane preparations, and also in *in vitro* outgrowth of goldfish retinal explants. Serotonin receptors are also expressed in amacrine cells and have been shown to modulate signal-processing in the mammalian retina. The broad range of physiological systems serotonin is known to be involved in include circadian rhythm, sleep-wake cycles, stress, cognitive function, and behaviour. In this study, distribution of a serotonin (5-HT) receptor polymorphism in myopes compared to emmetropes was carried out in an ethnically homogeneous population. Eighty-five unrelated myopes and eighty emmetropes were genotyped in this study. Myopes are Chinese Singaporeans with refractive errors of between -8.5 and -18.25D. Controls are ethnically and age-matched subjects with refractive errors of less than -0.50D. The MspI RFLP is a T/C polymorphism at nucleotide position 102 of the 5HTR2A gene on chromosome 13. It has been associated with schizophrenia and responsiveness to clozapine. We found an excess of the TT genotype for the controls but the difference was not statistically significant ($X^2=4.653$, $df=2$, $p\text{-value}=0.098$). There was also no major difference in allele frequency between the two groups. However, frequency of allele T was much higher in our Chinese population compared to populations of Western European descent. Further studies with other polymorphisms in this gene and other genes of the serotonin system would need to be carried out before the role of serotonin in the development of myopia can be elucidated.

Further investigation of the susceptibility locus for juvenile myoclonic epilepsy at chromosome 15q13-14. *N.L. Taske, F.V. Elmslie, M.P. Williamson, D. Curtis, M. Rees, M. Gardiner.* Paediatrics and Child Health, Royal Free and University College Medical School, The Rayne Institute, London, UK.

Juvenile myoclonic epilepsy (JME) is a common form of idiopathic generalised epilepsy (IGE), characterised by myoclonic jerks on awakening. There is evidence for a significant genetic component in its aetiology although the mode of inheritance remains uncertain. Significant evidence for linkage of JME to chromosome 15q13-14 (HLOD=4.4 at $\alpha=0.65$) was first demonstrated in a study of 34 JME families under the assumption of autosomal recessive inheritance (1). The maximum multipoint lod score at 1.7cM telomeric to marker D15S144 was originally thought to coincide with the locus for the gene encoding the $\alpha 7$ subunit of the neuronal nicotinic acetylcholine receptor (*CHRNA7*). However, subsequent description of the genomic organisation of *CHRNA7*, in addition to a refinement of the 15q13-14 physical map (2), has revealed that the *CHRNA7* gene lies approximately 2cM centromeric to D15S144. Furthermore, the *CHRNA7* gene was found to be partially duplicated with the duplicated region mapping to within 5cM centromeric of *CHRNA7*.

A further 6 marker loci spanning both the *CHRNA7* gene and the partial duplication have now been investigated in the 34 JME families originally described (1). Multipoint linkage analysis under the conditions described previously (1), revealed a maximum HLOD of 3.32 ($\alpha=0.53$; Zall= 2.96, P= 0.0004) at 2.04 cM telomeric to D15S217, in close proximity to the duplicated locus. Inspection of the haplotypes revealed that the region of chromosome 15 encompassing the duplicated locus (from D15S1043-D15S165) was identical by descent in one or both chromosomes of 22 out of 26 sibling pairs.

Transcripts from the partially duplicated *CHRNA7* gene have been detected in human hippocampus (2). It is not yet known if such transcripts are translated and as such, the function of the duplicated *CHRNA7* gene remains unclear. The potential role of the duplicated gene in JME is currently under investigation.

Literature cited: 1. Hum. Mol. Genetics 6:1329-1334; 2. Genomics 52: 173-185.

Dopamine beta-hydroxylase (DBH) linkage study in 65 multiplex bipolar families. *V.L. Willour, J.B. Potash, D.F. MacKinnon, S.G. Simpson, Y. Huo, J.R. DePaulo, M.G. McInnis.* Psychiatry Dept., Johns Hopkins University, Baltimore, MD.

Dopamine beta-hydroxylase (DBH), the enzyme that converts dopamine to norepinephrine, has been hypothesized to play a role in psychiatric illnesses. We studied DBH and flanking polymorphic markers in 65 bipolar multiplex pedigrees using parametric and nonparametric linkage analysis. Under a narrow phenotype definition, the maximum nonparametric lod score (NPL) is at DBH (NPL=1.85; $p=0.03$), and the peak maximum likelihood score (MLS) is approximately 2 centimorgans centromeric to DBH (MLS=2.85). Low DBH activity has been associated with psychotic depression. We identified a subset of 30 pedigrees with a psychotic proband with one or more psychotic first-degree relatives and repeated the analyses. The two-point LOD score generated for the dominant model increased from a maximum of 0.94 at theta 0.2 in the entire 65 pedigrees to a maximum of 2.64 at theta 0.045 in the 30 families enriched for psychosis. Close linkage to DBH was excluded in the 35 non-psychotic families. We hypothesize that variants at the DBH (or nearby) gene are associated with more severe forms of bipolar disorder.

Linkage Disequilibrium Mapping of Disease Genes by the Decay of Haplotype Sharing in Large Inbred

Populations. *J. Zhang*¹, *C. Ober*², *M.S. McPeck*¹. 1) Department of Statistics, The University of Chicago, Chicago, IL; 2) Department of Human genetics, The University of Chicago, Chicago, IL.

McPeck and Strahs (1999) propose a multipoint method for fine-scale linkage disequilibrium mapping. For populations with dependent recombinational histories, they suggest calculating a quasi-score estimating equation. They focus on the case when an exchangeable population model is assumed. We extend the method to the case of a Hutterite data set, consisting of 806 genotyped individuals who are related by a known 13-generation, 1623-member pedigree, for which exchangeability clearly does not hold. For this case, we consider a variation on the estimating equation approach taken by McPeck and Strahs (1999). We describe here a recursive algorithm to calculate the conditional correlation coefficients of ancestral-segment sharing for pairs of haplotypes in a large inbred pedigree, such as the Hutterites. From the conditional correlation and the estimating equations, we obtain an approximate log-likelihood of the sampled haplotypes, which can be used for mapping purposes. Reference: McPeck, M. S. and Strahs, A. (1999) Assessment of Linkage Disequilibrium by the Decay of haplotype Sharing, with Application to Fine-Scale Genetic Mapping. *Am. J. Hum. Genet.* 65:858-875.

Genome-wide scan of obesity genes in an African-American population. *X. Zhu¹, R.S. Cooper¹, A. Luke¹, G. Chen¹, A. Chakravarti²*. 1) Department of Preventive Medicine & Epidemiology, Loyola University Chicago, Maywood, IL; 2) Department of Human Genetics, Case Western Reserve University, Cleveland, OH.

Obesity is now beginning to replace undernutrition and infectious diseases as a significant contributor to human diseases. Several candidate regions have been identified by genome-wide linkage studies in different ethnic populations. No such study has been published yet from a black population. Therefore, we conducted a genome-wide scan among 1032 individuals in 202 African-American families in Maywood, IL, USA, collected as a part of the NHLBI Family Blood Pressure Program. We evaluated the evidence for linkage with body mass index using variance components analysis incorporated in GENEHUNTER. Our multipoint variance components analysis indicated that there was suggestive evidence for linkage in four genomic regions on chromosomes 2 (LS=1.4), 3 (LS=2.0), 5 (LS=1.85) and 10 (LS=1.84). The regions where linkage evidence was found on chromosomes 2, 5 and 10 in our study are consistent with studies in Mexican-American and French populations. The replication of linkage evidence using different phenotypes in different ethnic populations suggests that there may be common genes contributing to obesity-related phenotypes in populations with ethnically and environmentally distinct backgrounds.

Power to Reconstruct the Genotype of a Missing Individual. *N.D. Pankratz, D.L. Koller, T. Foroud.* Medical and Molecular Genetics, Indiana University, Indianapolis, IN.

To examine the power of various numbers and types of relatives to infer the genotypic data of a missing individual, we used GASP (Wilson et al, 1996) to generate three-generational pedigrees consisting of a pair of siblings, their deceased parents, and one of the siblings' spouse and three offspring. A range of simulation parameters (QTL effect size; overall trait heritability) were used to generate a quantitative trait to which various thresholds were applied to dichotomize the data. Markers with heterozygosity of 80% were simulated at 10 cM intervals. The gene was located in the middle of the marker map, 2 cM from the nearest marker. For each replicate, 500 families containing a pair of affected siblings in the second generation were retained for analysis. Genhunter-Plus (Kruglyak et al, 1996; Kong and Cox, 1997) was used to analyze the 500 families in each of 1,000 replicates.

Qualitative linkage analyses were performed with genotypic data assumed to be available for both affected siblings, the situation in which both members of the sibpair have DNA. This was our reference LOD score result, to which all subsequent analyses were compared. We then performed a series of steps in which we removed the affected sibling with the offspring and reanalyzed the sample, attempting to infer the missing individual's genotype using a sequentially smaller number of relatives. Six conditions were tested: 1) three offspring, with and without DNA on the deceased individual's spouse; 2) two offspring, with and without the spouse; and 3) one offspring, with and without DNA on the spouse. The lod score was recomputed under each condition in the 1,000 replicates consisting of 500 affected sibling pairs each. When the pedigree consisted of two or three offspring and the spouse or three offspring without the spouse, the analysis consistently retained over 70%; of the optimum lod score, over all simulation parameters. When one or two offspring and no spousal DNA or one offspring and the spouse were included, over 50%; of the optimum lod score was still obtained regardless of the simulation parameters.

Construction of a genetic marker map using genotypes from over 7500 Icelanders. *G.M. Jonsdottir¹, K. Jonasson¹, D.F. Gudbjartsson^{1,2}, A. Kong^{1,3}.* 1) Decode Genetics Inc., Reykjavik, Iceland; 2) Institute of Statistics and Decision Sciences, Duke University, Durham NC; 3) Department of Human Genetics, University of Chicago, Chicago, IL.

By using data from over 7500 Icelanders we have constructed a genetic map of more than 800 polymorphic markers. We have found some discrepancies in marker order in our genetic map compared to other published maps, specifically on chromosomes 2, 6, 8, 14 and 20. Our studies have been carried out with the aid of the program Crimap and our multipoint linkage program Allegro. To understand the discrepancies we plan to analyze the data of the publicly available CEPH-families and will report the results. Apart from marker order, we will also address the problem of estimating distances between markers with incomplete marker information. Comparison between Allegro and Crimap will be reported.

Novel mental retardation-epilepsy syndrome linked to Xp21.1-p11.4. *P. Heder¹, D. Alvarado¹, A. Beydoun^{1,2}, J.K. Fink^{1,2}*. 1) Department of Neurology, University of Michigan, Ann Arbor, MI; 2) Geriatric Research, Education, and Clinical Center, Veterans Affairs Medical Center, Ann Arbor, MI.

X-linked mental retardation is a clinically and genetically heterogeneous group of disorders some of which are associated with epilepsy. West syndrome (also known as Infantile Spasms Syndrome, X-linked, or ISSX) for example, mapped to Xp21.3-p22.1, is associated with mental retardation and infantile spasms. We evaluated kindred in which 8 normal sisters had a total of 12 sons, 7 of whom had both epilepsy and mental retardation. Seizures began at an average age of at 7.7 months (range 3 to 14 months) and were consistent with primarily generalized, tonic-clonic and atonic convulsions. Interictal electroencephalograms were normal. Mental retardation was moderate. Neurologic examinations were otherwise unremarkable with the exception of one affected subject who had generalized spasticity and mild ataxia. Electromyography and nerve conduction studies were normal in two affected subjects. Magnetic resonance imaging of the brain was normal in 4 affected subjects. There were no dysmorphic features. High resolution karyotype (one subject) and urine metabolic screen and plasma amino acids (another subject) were normal. We examined genetic linkage between the disorder and X-linked polymorphic microsatellite markers and identified tight linkage to a group of markers mapped to Xp21.1-p11.4. The maximum two-point LOD score +3.83 (penetrance 0.90, $Q = 0$) was obtained for markers DXS8090, DXS1069, DXS8102, and DXS8085. The locus spans 7.7 cM and extends between DXS1049 and DXS8054. Recently, Bruyere et al (1999) refined the West syndrome locus on Xp21.3-p22.1 to between DXS274 and AHC. The novel syndrome we describe, designated X-linked, Mental Retardation, Epilepsy (XMRE) differs clinically from West syndrome in seizure type (infantile spasms in West Syndrome; generalized tonic-clonic and atonic seizures in XMRE) and degree of mental retardation (profound in West Syndrome; moderate in XMRE). Moreover, the genetic locus for XMRE does not overlap that of West Syndrome. Identifying the XMRE gene will advance our understanding of mental retardation and epilepsy.

A novel X-linked mental retardation syndrome with short stature maps to Xq24. *E. Vitale¹, C. Specchia², M. Devoto², A. Angius³, S. Rong¹, K. Subramanian¹, M. Rocchi⁴, M. Schwalb¹, L. Demelas⁵, D. Paglietti⁵, S. Manca⁵, C. Mastropaolo⁵, G. Serra⁵.* 1) Dept. Microbiol & Mol Genetics, UMDNJ New Jersey Medical School, Newark, NJ; 2) Dept. Oncology, Biology and Genetics, University of Genova Italy; 3) CNR Molecular Genetics Alghero SS, Italy; 4) DAPEG Dept. Genetics University of Bari, Italy; 5) Inst. Child Neuropsychiatry University of Sassari, Sassari, Italy.

We describe a large family from Sardinia (Italy) that segregates a novel X-linked mental retardation (XLMR) syndrome. Eight males are affected over three generations. The phenotype observed in the eight affected males includes severe mental retardation (MR), lack of speech, coarse facies, distinctive skeletal features with short stature, brachydactyly of fingers and toes, narrow and down slanting palpebral fissures, large bulbous nose, hypoplastic ear lobe and macrostomia. Obligate female carriers are not mentally retarded, although some of them have mild dysmorphic features such as minor ear lobe abnormalities, as well as language and learning problems. Analysis of blood-lymphocyte karyotype and DNA analysis for fragile-X syndrome were normal. Linkage analysis for X-chromosome markers resulted in a maximum lod score of 3.61 with marker DXS1001 in Xq24. Recombinations observed with flanking markers identified a region of 16 cM for further study. Based on the clinical phenotype and these mapping data, we excluded other reported XLMR conditions. This evidence strongly suggests that the genetic disease in this family is unique.

Association between diabetes, obesity, glucose and insulin levels in the Old Order Amish and SNPs on 1q21-q23.

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The Old Order Amish (OOA) are a genetically homogenous founder population. Previous analysis of diabetes traits in the OOA revealed evidence of linkage and association with STR markers between 150-180 cM with the strongest evidence between 167-175 cM. 34 SNPs were genotyped in the 30 cM region. Association analysis between diabetes traits and the SNPs was conducted using the transmission disequilibrium software, TRANSMIT with individual SNPs and haplotypes composed of 2 to 4 SNPs. Two traits were analyzed, type2 diabetes (DM) and a broader phenotype of type2 diabetes or impaired glucose homeostasis (DM/IH). The samples consisted of 201 subjects in 25 pedigrees with 59 affected for DM and 374 subjects in 48 pedigrees with 125 affected for DM/IH. Association between the SNPs and the quantitative traits BMI, glucose and insulin levels during an OGTT was assessed using variance component methodology as implemented in SOLAR. This sample included 825 subjects in 28 pedigrees. Diabetic subjects were excluded from analysis of insulin traits. DM was significantly associated ($p < 0.05$) with 4 individual SNPs (POL965, 943, 1051 & 1061) from 155 to 180 cM. DM/IH showed association with 2 of these 4 SNPs (POL943 & 1051, 156 to 167 cM). The only haplotype associated with DM contained POL1051. None of the haplotypes involving POL965, 943, 1061 or other SNPs were significant. With DM/IH, two regions contained associated haplotypes. The first contained 4 SNPs from 157-161 cM while the other haplotype contained SNPs from 166.5 to 168 cM including POL1051. BMI showed significant association with SNPs between 161 to 168 cM. The majority of glucose and insulin traits with association lay between 167 to 179 cM. POL1051 showed association with BMI as well as with single glucose and insulin traits. POL907, 1057 & 1061 (168-179 cM) showed association with multiple glucose and insulin traits. In summary, our results strongly suggest that one or more diabetes or diabetes genes reside in this region.

The characterization and development of a highly informative mouse short tandem repeat (STR) marker set.

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To facilitate identification and selection of useful markers for a large number of commonly employed murine crosses, we developed and characterized a set of a highly informative short tandem repeat (STR) markers for mouse. Primer pairs for ~300 dinucleotide repeat markers were designed and typed against 47 inbred mouse strains. Markers were first selected to be maximally informative for the cross C57BL/6J x DBA/2J. Additional markers were selected to increase the density of the set and to increase informativeness for other crosses. Primer sequences for PCR were designed to allow multiplexing using the fluorescent dyes FAM, VIC, NED and ROX. The number of informative markers for the C57BL/6J x DBA/2J cross is currently 208 with an average spacing of ~7 cM. Information about the marker set is publicly available on the web at <http://www.cidr.jhmi.edu/mouse/mouse.html>. Investigators can submit a query for any pair-wise combination of inbred strains to retrieve the informative markers for a cross, or download a spreadsheet with the locus names, map positions, and allele sizes for each marker for all the strains tested. Detailed information averaged for all 47 strains can be accessed through the web site. Most of the markers have been typed against the C57BL/6J x *Mus spretus* (BSS) interspecific backcross panel (JAX) to anchor them to the existing mouse map and confirm their map order. Additional strains will be typed as their need is demonstrated by the mouse community. Similarly, markers may be added in the future to increase map density for the most frequently used crosses. *Mus spretus*.

Myotonic Dystrophy Type 2 (DM2) in Minnesota. *J. Day, C. Liquori, C. Johnson, A. Durand, L. Ranum.* Institute of Human Genetics, Minneapolis, MN.

We recently mapped a second myotonic dystrophy locus (DM2) to 3q21. DM2 has the same broad constellation of multisystemic clinical features as DM1. Although DM1 is caused by an untranslated CTG expansion, the pathogenic mechanism has been obscure. Mounting evidence supports pathogenic roles for DMPK, SIX5 and the elongated CUG transcripts. The striking clinical parallels between DM1 and DM2 suggest that these diseases share a common pathogenic pathway. Although the multiple mechanism theory is compelling when DM1 is considered alone, it is unlikely that a similar group of effects are duplicated at the DM2 locus. Detailed muscle histology and electrophysiology, ophthalmologic and endocrine evaluations have been performed to further define the clinical similarities between DM1 and DM2. Similar to DM1, individuals with DM2 had electrical myotonia (100%), distal weakness (81%), proximal weakness (59%) and muscle atrophy (14%). As in DM1, hyperinsulinemia (62%), hypercholesterolemia (50%), hypogammaglobulinemia (75%), primary testicular failure (89%) and modest CK elevation (80%) were common. Consistent with the distinctive biopsy findings of DM1, DM2 muscle also showed a combination of dystrophic muscle, non-necrotic fibers with a profusion of central nuclei, and severely atrophic fibers. On slitlamp examination all individuals had the type of cataract unique to myotonic dystrophy, with posterior subcapsular multicolored opacities. Muscle hypertrophy was evident in 9%, a feature not seen in DM1. Although no congenital or severely affected DM2 patients have been found, DM2 and DM1 share the same multisystemic features. In addition to the patients described above from the large DM2 family (lod=7.14 at q=0.00 for D3S1541), we have identified 18 DM1-negative families with myotonic dystrophy. Seven families large enough to analyze were all consistent with linkage to the DM2 locus. The identification of 18 families with DM2 (or possibly another novel form of DM) suggests that non-DM1 loci commonly cause myotonic dystrophy. In an effort to further understand the genetic heterogeneity and pathophysiology of the myotonic dystrophies we are using a positional cloning approach to identify the DM2 mutation.

Tibial muscular dystrophy - haplotype analysis of 150 Finnish patients and physical region of chromosome 2q31.

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Tibial muscular dystrophy (TMD) is an autosomal dominant late onset myopathy. The phenotype is relatively mild as muscle weakness manifests in the early 40s and remains confined to tibialis anterior muscles. We have assigned the locus of TMD to chromosome 2q31 using linkage analyses in four Finnish families. The critical chromosomal region is about 2 cM long consisting of markers cen-D2S148-D2S2173-D2S300-D2S385-D2S324-D2S2310-tel based on the obligatory recombinations in the families. The maximum multipoint lod score of 10.14 was obtained with marker D2S324. The purpose of this study was to narrow the linked region to be suitable for the successive disease gene identification and to analyze the genealogical background of TMD in Finland. A preliminary analysis of the physical region of interest is performed. In order to analyze haplotypes of TMD alleles we collected a study sample of 150 patients. Linked microsatellite markers were analyzed by standard methodology. YAC and PAC clones for all linked markers have been obtained. Haplotype analysis revealed the most common haplotype 9-4-4-8-2-8 in TMD patients. Total of 116 out of 150 disease alleles carried this haplotype. Some allelic variation was seen with the centromeric markers D2S148 and D2S2173 and telomeric marker D2S2310. All disease alleles carried a core haplotype of 4-8-2 of about 1 cM long. The core haplotype and three obligatory recombinations restricted the disease haplotype between the markers D2S2173 and D2S2310. TMD families show an uneven geographical distribution in Finland with clusters of cases on the western coast line and in eastern Savo-Karelia region. The same core haplotype was found from the disease alleles in both clusters evidencing for one founder mutation. It is noteworthy that the 300 kb gene for titin, a large structural protein of muscle, is located within the critical DNA- region making titin the most attractive candidate gene for TMD.

DOMINANT DEAFNESS IN PORTUGUESE FAMILIES-NEW MUTATIONS IN THE TECTA GENE. J.

Camara¹, H. Vieira¹, M. Vitorino¹, A. Mena¹, H. Caria¹, M. Simao², I. Galhardo², T. Netta², O. Dias², M. Andrea², C. Correia¹, G. Fialho¹. 1) Center of Genetics and Molecular Biology, University of Lisbon, Portugal; 2) Center of ORL, University of Lisbon, Portugal.

Mutations in the TECTA gene have recently been shown to cause a dominant non-syndromic prelingual form of hearing loss (DFNA8/12). In the present study, mutations in exons 14, 17 and 18 of the TECTA gene were searched in Portuguese families with a typical autosomal dominant pedigree and a prelingual deafness. In fact, these are the only exons where mutations have been described so far. The screening was carried out by SSCP analysis, and the PCR products presenting a mobility shift were bi-directionally sequenced. A new mutation was identified in exon 18, corresponding to a C to T transition in a codon for serine at nt 5901, and thus leading to a synonymous substitution (AGC-AGT). This mutation was found in the six affected members of one of the families under study presenting a moderate to severe hearing loss. On the contrary, it wasn't present in the three unaffected family members tested, as well as in 50 control unrelated normal-hearing individuals. Assuming that this mutation is the deafness-causing mutation, then these results would support the hypothesis, previously put forward by other authors, that a synonymous codon substitution may not be neutral as expected, but may cause a pathological phenotype. Ongoing studies will help to clarify this situation. Two unreported polymorphisms, IV17+40G-C and IV17+41G-A were identified in intron 17, when analysing the 50 individuals from the normal-hearing population, being their prevalence 20% and 14%, respectively.

MSX1 Mutation Search in Diverse Populations of Individuals with Clefting. *P. Jezewski¹, R. Schultz¹, A. Vieira^{1,4}, J. Machida³, Y. Suzuki³, A. Lidral², M. Johnson¹, B. Ludwig¹, S. Daack-Hirsch¹, S. O'Brien¹, R. Slayton¹, N. Natsume³, I. Orioli⁵, E. Castilla⁵, J. Murray¹.* 1) University of Iowa, IA; 2) Ohio State University, OH; 3) Aichi-Gakuin University School of Dentistry, Japan; 4) UFRJ and Bolsista da CAPES, Brazil; 5) UFRJ and FIOCRUZ, Brazil and CONICET, Argentina.

Cleft lip and/or palate (CL/P) and cleft palate only (CPO) are common nonsyndromic forms of clefting (1/500-1/2500 at birth). A syndromic form that includes tooth agenesis as well as variable penetrance and expressivity of clefting has recently been described in a Dutch family. This family had both multiple affecteds with multiple missing teeth (most commonly second premolars) as well as various forms of clefting. All affecteds were heterozygous for a (Ser104stop) mutation in the MSX1 gene. The MSX1 gene has previously been implicated in both CL/P and CPO by LD analysis. MSX1 has also been implicated in isolated tooth agenesis. We performed screens for similar mutations in three regions of the MSX1 gene. Exons 1 and 2 and a 410 bp region within the single intron showing significant homology between mouse and human were screened by direct sequencing of PCR products. Sequence analysis using PHRED/PHRAP/CONSED was used to identify sequence variants. The populations chosen for study included Iowa/Ohio, Filipino, Japanese, Vietnamese, and South American patients, all with clefts. To date 106 sequences from Exon 1, 130 sequences from Exon 2 and 130 from the intron have identified 8 previously identified variants, 1 new polymorphic variant and no clearly etiologic variants. Additional sequences will be completed on a minimum of 500 cases of NS CL/P or CPO but to date evidence suggests coding sequence mutations in MSX1 are rare causes of NS CL/P.

Detection of genomic variants of the LRP-gene and their importance for myocardial infarction. *S. Schulz¹, E. Archoukieh¹, U. Schagdarsurengin¹, P. Greiser¹, K. Werdan², I. Hansmann¹, C. Glaeser¹.* 1) Inst. of Human Genetics, Univ. Halle, Halle, Germany; 2) Dep. of Internal Med., Univ. Halle, Halle Germany.

LRP is a multifunctional receptor and one of the most interesting candidate genes for degenerative diseases such as atherosclerosis and Morbus Alzheimer. Material and methods: By use of a SSCP-analysis we screened in functional important domains of the LRP-gene for genomic variants in 214 patients undergone myocardial infarction (MI) as well as 224 healthy controls. Results: We detected four novel LRP-polymorphisms in the promoter (P C-25G) and in the ligand binding domain (E22C200T, I24C123A, I24G690A) as well as two mutations in the region responsible for clustering into coated pits (E88C118T, I88G15A). Investigating the P C-25G-polymorphism in the patient and control group we could not detect a person homozygous for the mutant allele. We found a protective influence of the mutant G-allele (CG-carrier) in the development of atherosclerosis considering age of onset of MI (<45 y. vs. >45 y.: 0.07 vs. 0.18, $p < 0.034$) as well as the ability to adaptation due to development of collaterals by angiogenesis and arteriogenesis (1- vs. 2- vs. 3-vessel diseases: 0.04 vs. 0.10 vs. 0.18, $p < 0.02$). This promotor-polymorphism resulted in the creation of a new GC-box, a binding site of the universal transcription factor Sp1. The three novel polymorphisms E22C200T, I24C123A and I24G690A were found to be associated. Investigating the genotype distribution in the patient and control group we found a significant decreased portion of the homozygous mutant-genotype TT-AA-AA among patients undergone MI (controls vs. patients: 0.11 vs. 0.03, $p < 0.009$), suggesting a protective effect of this genotype constellation on atherosclerosis. The two identified mutations in exon 88 and intron 88 of the LRP-gene could only be detected in one and four MI-patients, respectively. First reflections on functional consequences revealed that due to the mutation I88G15A a new acceptor splice motif is created which has a higher theoretical consensus value than the downstream original one. These results recommend the inclusion of the novel genomic variants of the LRP-gene in the individual coronary risk profile.

The development of TaqmanTM for the study of the pharmacogenetics of drug metabolizing enzymes. *D.J. Dow, B. Bahari, A.L. Knaggs, S.M. Lumsden, C.J. Allan, C-F. Xu, D.S. Montgomery, I.J. Purvis, M.S. Stubbins.* UK Discovery Genetics, GlaxoWellcome R & D, Building 2SA11, Park Road, Ware, Herts, SG12 0DP, UK.

GlaxoWellcome is committed to delivering the right medicine to the right patient and the study of pharmacogenetics (defined as the study of the variability in response to medicines due to hereditary factors) is one aspect of our current research programs which can have a major impact on this high-level objective. The most widely studied group of proteins within this research area are the Phase I and Phase II drug-metabolizing enzymes, including most notably, members of the cytochrome P450 (CYP) superfamily. We report here the development of more than 20 novel Taqman allelic discrimination assays to enable the medium throughput genotyping of known metabolic polymorphisms in a variety of Phase I and Phase II enzymes: CYP1A1, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP3A4, GSTP1, NAT1 & NAT2. The potential throughput of the Taqman assay is several thousand genotypes per person per day per Perkin-Elmer 7700 machine, limited only by individual laboratory PCR throughput. We also report several cost-limiting modifications to the standard allelic discrimination assay making it more affordable to both the industrial and academic community. The Taqman allelic discrimination assays are robust, requiring very little optimization and generating highly reliable and reproducible results. The assays developed here allow the rapid screening of samples from both clinical trials, and case-control association studies for pharmacogenetic effects. It remains to be seen to what boundaries the current platform can be stretched, but the capacity to multiplex these reactions would be an obvious advantage for the future. In conclusion, we present an extensive battery of cost-effective, robust, reliable, medium-throughput drug metabolizing enzyme genotyping assays to aid in our understanding of this increasingly important area of research.

Pharmacogenomic association by linkage disequilibrium mapping. *D. Katz¹, I. Chumakov², A. Cohen-Akenine², L. Bougueleret², C. Peponnet², N. Schork³, J. Drajek¹, M. Heath-Chiozzi¹, D. Cohen², M. Blumenfeld².* 1) Abbott Laboratories, Abbott Park, IL; 2) Genset SA, Paris, France; 3) Genset Corporation, La Jolla, CA.

We have completed a proof-of-concept experiment for genome-wide pharmacogenomic association studies. Zylflo[®] is a 5-lipoxygenase inhibitor approved for the treatment of asthma which induces hepatic transaminase elevations in a small subset (<5%) of patients during therapy. We searched for genetic differences between 89 cases, who had onset of hepatotoxicity (serum ALT³xULN) during or within 2 weeks after Zylflo[®] treatment, and 208 controls, who were treated with Zylflo[®] for at least 11 months without ALT elevations. SNPs were identified in 36 regions comprising somewhat less than 1% of the genome. Region choice was based on the presence of one or more candidate genes (47 total). SNPs were not specifically located within the candidate genes, but rather on BACs containing the candidate genes, to simulate conditions expected in a genome-wide case-control study based on linkage disequilibrium. These SNPs were then utilized to genotype cases and controls. In this study, there was strong evidence of association with hepatotoxicity for 2 candidate regions. There was lesser, possibly relevant, evidence of association for 4 additional candidate regions. These experimental results define a novel strategy to map genes for complex traits such as drug responses.

A 4 Mb Physical Map of a Type 2 Diabetes Mellitus Locus on Human Chromosome 1q. *J.K. Wolford, D. Blunt, C. Ballecer, V. Ossowski, C. Bogardus, M. Prochazka.* NIDDK/PECRB, NIH, Phoenix, AZ.

We have previously identified a locus for diabetes susceptibility on chromosome 1q21-q23 in the Pima Indians. Linkage to diabetes in the same chromosomal interval has also been reported in the Old Order Amish and in Caucasians living in Utah. To facilitate linkage disequilibrium mapping and positional cloning of the disease gene, we have constructed a physical map encompassing the peak of linkage in the Pimas at marker D1S1677. Using PCR screening of commercially available human genomic libraries with either known STSs or markers generated in our laboratory, and in silico searches for clones mapped to this region, we have identified over 600 bacterial artificial chromosome (BAC) and P1-derived artificial chromosome (PAC) clones throughout the ~30 cM linkage interval. To specifically map the region underlying our peak of linkage, we have generated a complete 4 Mb sequence-ready contig of 225 overlapping BACs flanked by markers D1S484 and D1S196. BAC clones in this interval have been ordered by STS content mapping and the extent of overlap confirmed by fluorescence-labeled fingerprinting. The average BAC insert size is 135 kb and the minimum tiling path consists of 50 clones. This region is gene-rich and our physical map has allowed accurate placement of 27 anonymous ESTs and 14 genes previously mapped by radiation hybrid to 1q, including 4 potential candidate genes: APOA2, RXRG, PBX1, and USF1. At present, we are utilizing this physical map to identify a set of single nucleotide polymorphisms (SNPs) for use in linkage disequilibrium mapping of the putative diabetes locus. We have identified over 200 SNPs throughout the entire linkage interval and found nominal associations of type 2 diabetes with 2 SNPs ($p=0.0003$). However, because these markers do not appear to account for the linkage, we are currently in the process of identifying SNPs at approximately 50 kb intervals throughout the 4 Mb region to systematically refine the position of the disease locus.

Identification of novel SNPs in cocaine amphetamine regulated transcript (CART) gene: a candidate gene for anorexia nervosa or obesity. *K.M.O. Brown¹, H. Eastwood¹, N. Bodsworth², D.A. Campbell², L.F. Pieri³.* 1) Molecular Medicine Unit, Leeds University, Leeds, U.K; 2) SmithKline Beecham Pharm, Harlow Essex, U.K; 3) Yorkshire Centre for Eating Disorders, Seacroft Hospital, Leeds, U.K.

There is significant evidence for genetic factors in the susceptibility to anorexia nervosa (AN) and obesity. Knock-in mice who over-express CART exhibit decreased appetite. CART protein localizes to areas of the brain involved in regulating feeding behavior and various metabolic functions. The current study focused on identifying SNPs in CART. The genomic structure of CART has been determined and contains 3 exons producing a 796bp transcript. PCR primers were designed to produce fragments of approximately 350bp in size covering the coding region of the gene and intron-exon boundaries. Potential SNPs were identified by analysing the resultant PCR products by dHPLC (Transgenomic WAVE). Putative SNPs were confirmed by automated sequencing. Analysis of the coding region of CART failed to identify any SNPs. However, we have identified two SNPs within the 3UTR of the gene resulting in a G to A substitution at 1513bp (G1513A) and a C to A at 1562bp (C1562A). We will extend this analysis to the putative promoter and other non-coding regions of the gene in order to identify further variants within this gene. The polymorphisms described above will allow us to examine the role for this gene in the genetic predisposition to AN and or obesity.

A new polymorphism in the Hermansky-Pudlak syndrome gene HPS-1; relevance for mutation detection. *D.L. Fitzpatrick¹, M. Rausche¹, M. Huizing¹, C. Castellon², G. Donazzan², W.A. Gahl¹.* 1) Section on Human Biochemical Genetics, Heritable Disorders Branch, NICHD, NIH, Bethesda, Maryland 20892; 2) Departments of Clinical Genetics and Pulmonology, General Hospital, Bolzano, Italy.

The HPS-1 gene is the first gene found to be responsible for the autosomal recessive disorder Hermansky-Pudlak syndrome (HPS). HPS is characterized by oculocutaneous albinism, a platelet storage pool deficiency, and ceroid lipofuscinosis. The HPS-1 gene, on chromosome 10q23, encodes a 79 kDa protein of unknown function with no homology to any known protein. In performing mutation detection of HPS patients, we found a homozygous T217C (Val4Ala) substitution in exon 3 of HPS-1. At least 3 Val to Ala substitutions, in other genes, are known to be disease-causing mutations. However, in screening 88 controls for the T217C missense by RFLP and sequencing analysis, we found 3 normal individuals who were heterozygous for the base change. Moreover, screening of family members of the HPS proband revealed an unaffected sibling who was homozygous for the missense. We conclude that this Val4Ala substitution is a polymorphism in the HPS-1 gene, joining several other known polymorphisms in HPS-1, including 4 other amino acid substitutions. Interestingly, there have been no disease-causing missense changes in HPS-1 described to date. Our findings are important for future HPS-1 mutation detection.

Evidence for genetic modifiers of cystic fibrosis lung disease. *R.J. Marsick, M.L. Drumm.* Departments of Pediatrics and Genetics, Case Western Reserve Univ, Cleveland, OH., USA.

Inflammation contributes to lung deterioration in CF patients, and ultimately to patient mortality. Polymorphisms in promoter regions of the interleukin-10 (IL-10) and tumor-necrosis factor-alpha (TNF-alpha) genes contribute to differential levels of protein production. An A at the 1082 position of the IL-10 promoter is associated with decreased IL-10 production (Turner et al., 1997), while an A at the 308 position of the TNF-alpha promoter is associated with increased TNF-alpha production (Wilson et al., 1997). IL-10 is an anti-inflammatory cytokine, whereas TNF-alpha acts in a pro-inflammatory manner, predicting that genetic variants leading to lower levels of IL-10 and higher levels of TNF-alpha would confer a heightened inflammatory response. We found a significant association between the IL-10/TNF-alpha genotype combination (AA/AA or AA/AG) predicting a heightened inflammatory response and decreased pulmonary function in CF patients homozygous for the DF508 mutation, but no associations were found for either gene alone. We genotyped 62 DF508 homozygous CF patients for the IL-10 and TNF-alpha polymorphisms, and identified 4 patients with the AA/AA or AA/AG IL-10/TNF-a genotype combination. These patients had an average FEV1 predicted of 85.2% between the ages of 5-7 years, 79.3% between ages 9-11, and 70.4% predicted between ages 14-16. Patients without this genotype combination averaged 109.3%, 89.4%, and 80.0% predicted in the respective age groups. There was no difference between the rates of decline (slope) in each of these groups. These results imply an important interaction between cytokines that contribute to CF pulmonary disease and warrant investigation into other polymorphic inflammatory mediators.

Statistical Considerations for Genome-Wide Scans: Design and Application of a Novel Software Package

POLYMORPHISM. *T. Niu*^{1, 2}, *B. Struk*^{2, 3}, *K. Lindpaintner*^{2, 3}. 1) Prog Population Genetics, Harvard Sch Public Health, Boston, MA; 2) Cardiovasc Div, Dept of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 3) Max Delbrück Center for Molecular Medicine, Berlin, Germany.

Genome-wide scans for linkage remain as an important tool in identifying genes underlying polygenic traits. Two specific problems associated with the optimization of these study designs are the questions: (i) how to best estimate the important parameters in conducting a two-stage genome scan using full sib pairs and incompletely informative microsatellite markers; and (ii) what type of single nucleotide polymorphisms (SNPs) are most effective in conducting a SNP-based genome scan. To address these questions, we designed a novel software package called POLYMORPHISM. This program is designed to calculate linkage parameters for both single-point and two-point settings. In single-point analysis, the heterozygosity (HET), polymorphism information content (PIC), and linkage information content (LIC) statistics based on marker allele frequencies are calculated. In two-point analysis, joint PIC values for two markers, the conditional probability of detecting linkage phase (P), the frequency of double heterozygotes (H), and the expected number of informative meioses (ENIM) are calculated. We applied this program in a CEPH pedigree-derived genotyping dataset, and based on the results, we concluded that: (a) LIC values are crucial for getting accurate estimates on those parameters that are important for a two-stage genomic screening; and that (b) to optimize the cost-effectiveness of a SNP-based genomic screening, a balance between marker information content and marker density can be modeled.

A protective association of the HLA-DR*0103 polymorphism with Rheumatoid Arthritis. A. Milicic¹, D. Lee¹, M. Brown¹, C. Darke², K. Mackay¹, B. Wordsworth¹. 1) Wellcome Trust Centre for Human Genetics, Oxford, UK; 2) Regional Tissue Typing Laboratory, Cardiff, UK.

Rheumatoid Arthritis (RA) is strongly associated with several variants of the highly polymorphic HLA-DRB1 locus, primarily the members of the family of DRB1*04 (DR4) and DRB1*01 (DR1) alleles. These encode HLA molecules with a similar linear amino acid sequence around the antigen binding-site, which has led to the "shared epitope" hypothesis of susceptibility to RA. In contrast, certain DR4 alleles (DRB1*0402, *0403, *0407) confer protection against RA and it has been suggested that DRB1*0103 may also be protective. Some studies also suggest that DRB1*01 homozygotes may be under-represented among RA patients. This study aimed systematically to ascertain the association of specific DRB1*01 alleles with RA.

668 British Caucasian RA patients were typed for the common DRB1 alleles using sequence specific PCR (SS-PCR). The data were compared with 13,634 normal British Caucasian controls. Statistical analysis was carried out using the relative predispositional effect method (RPE) which enables the relative effect of one allele, independent from other alleles, to be determined. The strong association with DRB1*04 was confirmed ($OR=3.5$, $p<10^{-10}$) and a strong positive association, independent of DR4, detected with DRB1*0101/02 ($OR=3.0$, $p<10^{-10}$). DRB1*10 also showed positive association ($OR=2.8$, $p<0.0003$) and DRB1*09, which has previously been reported only in Chilean RA patients, was significantly increased among these patients ($OR=2.0$, $p<0.003$). The frequency of DRB1*01 homozygotes in the patient cohort corresponded perfectly to the Hardy-Weinberg prediction and was not decreased. A statistically significant decrease in the frequency of the relatively uncommon DRB1*0103 allele among the patients was noted ($OR=0.2$, $p<0.036$).

This study reveals a strong negative association of DRB1*0103 with RA. This allele shows the same antigen binding-site sequence as DRB1*0402, which is also negatively associated with RA. This highlights the crucial role this sequence plays in predisposition to RA, whether in the context of a DRB1*01 or DRB1*04 genetic background.

Arrayed Primer EXtension (APEX) as single nucleotide polymorphism (SNP) scoring technology. A. Kurg¹, E. Lõhmussaar¹, T. Nikopensius¹, N. Tõnisson², A. Lushnikov¹, I. Kask¹, J. Zernant², H. Pavel², A. Metspalu^{1,2}. 1) Institute of Molecular & Cell Biology, University of Tartu, Estonian Biocentre, Tartu, Estonia; 2) Asper Ltd., Tartu, Estonia.

SNP-s are being handled as the markers of choice for genome wide association mapping. We present Arrayed Primer Extension (APEX) technology applied for scoring of SNP-s all over the genome. APEX is a resequencing method for identification of different types of sequence alterations. An integrated system with chip and template preparation, multiplex primer extension on the array, fluorescence imaging equipment and data analysis is described. The method is based upon a two dimensional array of oligonucleotides, immobilized via their 5'end on a glass surface. The DNA sample is amplified by PCR, digested enzymatically, and then annealed to the immobilized primers, which promote sites for template-dependent DNA polymerase extension reactions using four fluorescently labeled dideoxy nucleotides. The GenoramaTM CCD camera-based four-channel fluorescence imaging system and genotyping software is used for semiautomatic analysis of the assay. In the present study APEX technology was successfully used for SNP-based genotyping. We have selected 68 SNPs over the whole genome and estimated the allele frequencies and heterozygosities of these SNPs in Estonian population analyzing 160 chromosomes. From the 68 analyzed SNPs 58 were polymorphic according to allele frequencies data. The 50 analyzed SNPs, with allele frequencies (0.4- 0.5) were used for paternity testing and human identification. The usage of SNPs for paternity testing allows for higher power of exclusion than the current STR-based method. The results demonstrate the feasibility of the APEX chips in SNP scoring. In addition, the system is a good platform for developing of larger SNP scoring chips, which could be used for genome wide association mapping by linkage and linkage disequilibrium (LD) studies.

Single nucleotide polymorphism discovery in candidate genes for rheumatoid arthritis. *C. Hacker¹, D. Thomas¹, J. Doshi¹, R.P. Kimberly², T.R. Gingeras¹, N. Patil¹.* 1) Affymetrix, Santa Clara, CA; 2) Division of Clinical Immunology and Rheumatology, UAB Arthritis and Musculoskeletal Center, University of Alabama at Birmingham, Birmingham, AL.

Rheumatoid arthritis (RA) is a chronic inflammatory disorder that affects 1% of the adult population in the United States. A complex interaction of genetic factors is most likely involved in the development and progression of RA. Using GeneChip™ probe arrays designed to detect single nucleotide polymorphisms (SNPs), we have screened a subset of 15 candidate genes, covering 128 kb of human genomic DNA, for SNPs in 30 patients with RA and 30 unaffected individuals. These candidate genes were chosen based on biochemical evidence suggesting their importance in inflammatory disorders and have been shown to encode proteins involved primarily in the immune response and apoptosis. Preliminary results show a number of novel SNPs in the coding and non-coding regions of the IL-4, IL-15, and IL-1 receptor antagonist genes. In addition to SNP patterns, we are interested in defining differential gene expression profiles in individuals affected and unaffected with RA. We hope to couple our present SNP data with an analysis of gene expression patterns. Our goal is to build genotype-phenotype relationships by associating SNP and gene expression patterns in patients with RA.

Allelic frequencies and patterns of single nucleotide polymorphisms in candidate genes for asthma and atopy in Iceland. *H. Hakonarson¹, E. Ostermann¹, U.S. Bjornsdottir², T. Arnason¹, S.A. Gudnadottir¹, J. Gulcher¹, K. Kristjansson¹, T. Gislason², D. Gislason², K. Stefansson¹.* 1) deCode Genetics, Inc, Reykjavik, Iceland; 2) Division of Allergy and Pulmonary Medicine, Vifilstadir University Hospital, Iceland.

While numerous asthma and atopy loci have been reported in studies demonstrating linkage of the asthma-associated phenotypes, atopy, elevated IgE levels, and bronchial hyperresponsiveness to microsatellite markers and single nucleotide polymorphisms (SNPs) within specific candidate asthma and atopy genes, most of them lack statistical power for significance. We assessed the nature, pattern and frequency of SNPs in 23 candidate genes for atopy and asthma in Iceland and examined for linkage to these genes. We sequenced approximately 10 kb of genomic sequences comprising selected promoter regions and exons from 94 atopic asthmatic patients and 94 controls (i.e., 376 alleles total) or approximately 3.6 Mb total of genomic DNA, isolated from peripheral blood mononuclear cells. Linkage analysis was also performed using region-specific markers for these 23 candidate genes. We identified 36 candidate SNPs with an average minor allele frequency of 20.5%. Twenty three (64%) were within the coding sequences, and 11 (48%) of those lead to a predicted change in the protein sequence. In general, the frequencies of the major protein-altering SNPs were comparable to those reported in other atopy and asthma populations of European descent. No differences were detected in the allelic frequencies of SNPs in any of these 23 candidate atopy and asthma genes or their combinations between controls and the atopic asthmatic patients. Moreover, linkage analysis that included over 90 Icelandic families with a total of 269 atopic asthmatic patients, uncovered no evidence of linkage to markers associated with any of these 23 candidate atopy and asthma genes. We conclude that it is unlikely that these 23 genes commonly recognized as "candidate" atopy and asthma genes significantly influence the expression of the atopic asthmatic phenotype or that genetic variations within these genes contribute to the susceptibility of atopic asthma.

A Candidate Gene Study of Attention Deficit Hyperactivity Disorder utilizing computationally identified SNPs and a High-Throughput Fluorescent Polarization SNP genotyping assay. *V. Kustanovich¹, L. Crawford¹, S.*

Kalman², K. Irizarry³, N. Brown⁴, C. Lee³, S. Smalley⁵, S. Nelson¹. 1) Human Genetics, UCLA, Los Angeles, CA; 2) LJI Biosystems, Inc., Sunnyvale, CA; 3) Molecular Biology Institute, UCLA, Los Angeles, CA; 4) Computer Science, UCLA, Los Angeles, CA; 5) Psychiatry and Biobehavioral Science, UCLA, Los Angeles, CA.

Purpose: To identify susceptibility loci contributing to attention deficit hyperactivity disorder (ADHD) in a large family-based population sample by large-scale SNP discovery and high-throughput genotyping

Methods: SNPs were identified by a computational approach utilizing Bayesian estimates of error-rate within publicly available EST data sets (Irizarry et al, in press). This database forms a resource from which we have identified thousands of SNPs, which will be useful in our analysis of complex neurobehavioral traits. Further, we have worked to optimize a fluorescence polarization SNP assay for high-throughput genotyping of SNPs on our ADHD sample. We have found that this method is highly accurate, cost-effective, homogeneous and fast.

Results: Attention deficit hyperactivity disorder is a common neurobehavioral disorder with early-onset in which impulsive, hyperactive, or inattentive behaviors lead to impairment in school, home, or social functioning. Although the genetic basis of ADHD is clear, attempts at identification of major susceptibility loci have proven refractory. Our group, as well as others, had found weak associations with genes in the dopamine and catecholamine pathways. In addition to a genome-wide scan which is currently in progress, we are analyzing SNPs in candidate genes in ADHD. We have identified hundreds of SNPs in candidate genes. We are particularly interested in the subset of common SNPs, which encode non-conservative amino acid alterations in brain-expressed genes. These likely functional variants will be tested as direct susceptibility alleles in ADHD. We have gathered over 250 families with 492 affected subjects. The large resource of the UCLA SNP database and the power of high-throughput SNP genotyping will allow us to test many candidate polymorphisms in ADHD.

Netherton's gene polymorphism and serum IgE levels in a general population sample. *M.F. Moffatt¹, K.C. Wong¹, A. James², A.J. Walley¹, A.W. Musk², W.O. Cookson¹*. 1) WTCHG, University of Oxford, Oxford, Oxfordshire, UK; 2) Department of Respiratory Medicine, Sir Charles Gairdner Hospital, Perth Western Australia.

Asthma and eczema (atopic dermatitis) are part of the syndrome of Atopy, and are characterised by Immunoglobulin E (IgE) responses to common allergens. Netherton's disease is a rare recessive skin disorder in which atopy is a universal accompaniment. Netherton's disease has recently been shown to be due to a defect in the SPINK5 gene. SPINK5 is on epithelial and mucosal surfaces and in the thymus. It encodes LEKTI, which is a fifteen-domain serine proteinase inhibitor. We have previously shown that a 420Glu->Lys polymorphism within the gene is associated with atopy, asthma and eczema. In order to further investigate this association we have genotyped 420Glu->Lys and 6 adjacent SNPs in exons 13 and 14 of the gene in 1004 subjects in 230 families from an Australian general population sample. Six thousand four hundred and nine genotypes were analysed for association using a modified Monks test implemented in the QTDT program. Empirical significance levels were calculated by Monte-Carlo simulations. 420Glu->Lys showed significant association with the total serum IgE ($p=0.016$). The coding polymorphisms 368Asn->Ser ($p=0.010$) and 386Asp->Asn ($p=0.027$) and the intronic SNP 221-50G->A ($p=0.008$) showed similar associations. However the silent polymorphism 118C->T ($p=0.0000$) showed a much more significant association. Two-point linkage disequilibrium (LD) estimations between the markers were measured after haplotypes were constructed using the SIMWALK2 program. LD was high between all the markers ($D' \gg 0.9$). Three marker combinations accounted for 92% of all haplotypes, but did not show significant association with the IgE concentration. The results suggest that 420Glu->Lys and the other coding polymorphisms may not be disease-causing. It also seems that disease-causing alleles are not exclusively present on the common haplotypes formed by these markers. *MFM & KCW contributed equally to this work.*

An efficient SNP discovery procedure used in our association studies of rheumatoid arthritis. *R. Yamada*^{1, 2}, *T. Tanaka*¹, *Y. Ohnishi*¹, *K. Yamamoto*², *Y. Nakamura*¹. 1) Laboratory of Molecular Medicine, Human Genome Center, IMS, U of Tokyo, Tokyo, JAPAN; 2) Dept. of Allergy and Rheumatology, U of Tokyo, Tokyo, JAPAN.

Rheumatoid arthritis (RA) is one of the relatively common diseases with considerable genetic contribution to its pathophysiology. For the dissection of its genetic background, examination of single nucleotide polymorphisms (SNPs) is a promising method. Since genetic contribution of each gene to RA is expected small, a large number of SNPs need to be investigated. For this kind of researches, an efficient method to discover SNPs is required in the first place. We report a method to discover SNPs with relatively low false positive rate, which were verified by hybridization of allele specific oligonucleotide (ASO) method in our association studies of RA. Our SNP discovery method was as follows: Genomic DNA from 48 individuals were used to prepare 16 mixtures of 3 individuals DNA. Using the 16 DNA mixtures as template, target regions were amplified and directly sequenced. The sequence chromatograms were processed by PolyPhred, an SNP-discovery supporting program, to detect SNP candidates. The chromatograms were inspected by experienced researchers to verify the candidates before confirmation. For validation of SNP-candidates, ninety-six cases and 96 controls were genotyped for 46 SNP candidates with ASO method. Among the validated SNPs the lowest minor allelic frequency calculated based on ASO-genotyped data was 0.047. Only three out of 46 ASO experiments for SNP candidates failed to separate samples into different genotype groups, which may be due either to false positive results or to the failure of ASO experiments. The false positive rate of our SNP discovery method (at most $3/46=0.065$) seemed adequately low to proceed to the genotyping step without further verification step in large-scale genotype-based study where a small fraction of false positive SNP candidates was considered negligible.

Identification of a novel *HinfI* RFLP of the interleukin-1 type 1 receptor gene (IL1R1): linkage to type 1 diabetes and correlation to IL-1R1 plasma level. *F. Pociot, R. Bergholdt, Z.M. Larsen, N.A. Andersen, O.P. Kristiansen, J. Johannesen, J. Nerup.* Steno Diabetes Ctr, Gentofte, Denmark.

The human interleukin-1 type I receptor (IL-1RI) (MIM 147810) is the signal transducing receptor for IL-1, a principal proinflammatory cytokine, which is cytotoxic to pancreatic islet beta cells. The IL-1R1 gene, IL1R1, maps to chromosome 2q12. The 5'UTR includes exon 1A, exon 1B and exon 1C of which only one is transcribed partly dependent on stimulus and cell type. We have previously reported association of a IL1R1 promoter *PstI* RFLP with type 1 diabetes mellitus (T1DM), but subsequently no linkage was demonstrated. In the present study, we have further characterized the promoter region demarcating exon 1B and 1C by sequencing and mutation scanning. New sequence was obtained 2118 bp upstream and 1608 bp downstream this region. Within this sequence, we identified three frequent single nucleotide polymorphisms (SNPs). PCR-based RFLP assays were established and the 3 polymorphisms were typed in a Danish T1DM family collection comprising 103 simplex and 150 sib-pair affected families. Linkage was evaluated by the sib-TDT (transmission disequilibrium test). One of the polymorphisms, defined by a *HinfI* RFLP assay, demonstrated linkage to T1DM, $p(\text{sTDT}) = 0.026$. Random transmission was observed to unaffected offspring from heterozygous parents, $p = 0.87$. No evidence for positive linkage was seen for the other tested polymorphism, $p = 0.14$ and $p = 0.21$, respectively. To evaluate the possible functional significance of the *HinfI* polymorphism, we measured circulating IL-1R1 plasma level in 30 T1DM patients and in 30 control subjects ten with each genotype in both groups. Significant differences in plasma levels in relation to genotype were found for diabetic patients ($p = 0.049$), whereas no such difference was observed in control subjects ($p = 0.58$). In the diabetic group, the +/+ genotype correlated with the highest IL-1R1 plasma level, whereas the plasma levels were comparable for the +/- and -/- genotypes.

A Novel Method for Estimation of Short Tandem Repeat Polymorphic Marker Allele Frequencies from Pooled DNA Samples. *K. Wang*¹, *T.A. Braun*², *V.C. Sheffield*^{3,4}. 1) Dept Biostatistics, Univ Iowa, Iowa City, IA; 2) Dept Genetics, Univ Iowa, Iowa City, IA; 3) Dept Pediatrics, Univ Iowa, Iowa City, IA; 4) HHMI.

Linkage disequilibrium analysis is an important method for mapping disease genes in complex disorders. Traditionally, the subjects in a study are genotyped individually. A much more efficient alternative is to use DNA pooling, in which the DNA samples from cases and controls are pooled into two separate pools (Arnheim et al., 1985; Sheffield et al., 1995), and estimate allele frequencies from the pooled samples. For short tandem repeat polymorphisms (STRPs), the allele frequencies can be estimated from a quantification of the gel image of the pooled PCR products of the DNA samples. In the literature, estimation of allele frequencies are based on either the height of the corresponding peaks or the area under the corresponding peaks. The estimation involves the use of arbitrary parameters and are inherently biased due to the overlapping of different alleles on the gel image. Here, we adopt a mixture model approach. This method explicitly models the overlapping effects. The allele frequencies are estimated using the EM algorithm. This method eliminates some of the arbitrariness associated with the existing methods. Analysis of 3 tetra-nucleotide markers on 88 cases and 176 controls indicates this method produces better estimates of the allele frequencies.

Association studies of the polymorphisms in NRAMP1 gene with Behcet's Disease. *R.M. Toydemir^{1,2}, P. Bayrak Toydemir¹, A. Gurler³, I. Bokesoy¹, K.P. Chang².* 1) Dept Medical Biology, Medical Genetics Division, University of Ankara, Faculty of Medicine, Ankara, TURKEY; 2) Dept Microbiology and Immunology, FUHS, Chicago Medical School, North Chicago, IL; 3) Dept Dermatology (Behcet Center), University of Ankara, Faculty of Medicine, Ankara, TURKEY.

Natural resistance associated macrophage protein 1 (NRAMP1) is a macrophage protein, which functions as an iron transporter. It has pleiotropic effects on macrophage function and T cell polarization via inducible nitric oxide synthase, tumor necrosis factor alpha, interferon gamma, and MHC Class II expression. Several polymorphisms in this gene have been shown to be associated with susceptibility to intramacrophage parasites as well as autoimmune disorders, such as rheumatoid arthritis. Named after Dr. Hulusi Behcet, Behcet's Disease (BD) is an inflammatory disease of unknown etiology. The major characteristics of this multisystemic disease include orogenital ulcerations, uveitis, thrombophlebitis, vasculitis, arthritis, and central nervous system involvement. Several possibilities in etiopathogenesis are being evaluated and there is evidence to support the role of immune dysfunction. Abnormal T cell polarization and dysfunction of macrophages are two of the consistent findings in patients. Therefore, NRAMP1 gene may have a role in the pathogenesis of BD. We evaluated the effects of NRAMP1 polymorphisms in 60 BD patients and 100 healthy individuals using polymerase chain reaction followed by restriction fragment length polymorphisms. We did not find any significant difference in the frequency of NRAMP1 gene polymorphisms between patient and control groups. Further analysis of flanking regions of NRAMP1 gene may show significant association with BD.

Developing a SNP map of the human genome. A.K. Voltz¹, A. Braun², P.S. Chines³, K.F. Doheny⁴, M.S. Hilliard¹, D.P. Little², P.D. Witmer⁴, R.L. Nussbaum¹. 1) DIR/IDRB/NHGRI, NIH, Baltimore, MD; 2) Sequenom, Inc., San Diego, CA; 3) DIR/GMBB/NHGRI/NIH, Bethesda, MD; 4) CIDR, JHU, Baltimore, MD.

We are developing a single nucleotide polymorphism (SNP) map of the human genome to identify genes and mutations that contribute to genetically complex diseases such as heart disease, cancer, asthma and mental illness. The SNPs are retrieved from public databases or provided by collaborators. Although there are more than 135,000 SNPs in dbSNP, most of these have been predicted from DNA sequence data only, with no further experimental validation. For our SNP map, we select only those SNPs that have demonstrated Mendelian inheritance in families or been observed experimentally within a population. This strategy should reduce the number of false SNPs arising from errors in DNA sequencing techniques. Our SNPs are scored experimentally using Sequenom's Allelic Discrimination by Primer Extension MALDI-TOF Mass Spectrometry method. PCR primers are designed using Primer3 (Whitehead). In addition to PCR primers, another oligonucleotide (the EXTEND primer) is needed for SNP detection. We developed a program that designs EXTEND primers on both forward and reverse DNA strands. Primer extension reactions will be multiplexed so that 10 SNPs can be assayed simultaneously within a single individual. To ensure that SNPs are unique, we do "in silico" mapping of SNPs. A modified version of e-PCR (NCBI) is used to map SNPs to finished human genome sequence. In addition to eliminating redundant SNPs, "in silico" mapping helps to avoid SNPs that map to more than one location within a chromosome. "In silico" mapping of SNPs to finished genomic sequence also provides a common ground for ordering SNPs within a chromosome. We have produced SNP maps for human chromosomes 22 and 21. Our chromosome 22 SNP map currently contains 100+ unique SNPs within 34 Mb of finished genomic sequence, at an average spacing of 340 kb. Although the ref SNP map available at NCBI contains over 5000 SNPs on chr22, these SNPs are unvalidated. We are expanding our SNP data mining to other chromosomes as finished sequence becomes available.

Combined DNA Index System (CODIS), for the microsatellite analysis using the infrared technology.

Comparison with the UV-based technology. *U. Ricci, I. Sani, S. Guarducci, V. Lazzerini, S. Pelagatti, A. Brusaferrì, E. Lapi, A. Cecconi, M. Lapini, L. Giunti, M.L. Giovannucci Uzielli.* University of Florence, Department of Paediatrics, Azienda Meyer, Genetics and Molecular Medicine Unit, Florence Italy.

In 1997, a new Combined DNA Index System (CODIS) was published by FBI, especially designed for genetic personal identification. The core of this system is represented by a battery of 13 Autosomal DNA Polymorphic Markers, useful not only for forensic genetics, but also for Medical Genetics problems. CODIS, used in the United States, contains all the STR loci currently recommended by the European Network of Forensic Science Institute (ENFSI) DNA Working Group. The CODIS core includes the following STR, DNA Markers: TPOX, D3S1358, FGA, CSF1PO, D5S818, D7S820, D8S1179, TH01, vWA, D13S317, D16S359, D18S51, D21S11, and the X-Y homologous amelogenin genes. We set up a new test for the analysis of the CODIS core, by using four multiplex PCR amplification reactions, and the IR automated fluorescence monolaser sequencer LICOR-4200. Blood stains, semen, saliva, hairs, teeth, bones and paraffin embedded tissues, were analyzed. The results obtained with our method were compared with an UV-based automated sequencer and an UV capillary sequencer that used a commercially available kit. Thirty undegraded DNA samples and twenty stains were analyzed. The results are completely in agreement, suggesting the possibility to exchange data between laboratories that use different technologies.

Complete mutation scanning of the coding (cDNA) and minimal promoter region of an IDDM candidate gene

SEL-1L. Z. Larsen¹, M. Zollo², I. Biunno³, M. Cattaneo³, J. Nerup¹, F. Pociot¹. 1) Steno Diabetes Center, Gentofte, Denmark; 2) TIGEM, Milano, Italy; 3) Istituto Tecnologie Biomediche Avanzate del CNR, Milano, Italy.

SEL-1L is highly similar to the *C. elegans* sel-1 gene, an important negative regulator of the notch pathway, which acts as a key regulator of the cellular proliferation and specification processes in both vertebrates and invertebrates. *SEL-1L* is abundantly expressed in the pancreas (both acini and the insulin producing b-cells) of healthy individuals, whereas low to undetectable levels are observed in other adult and in some fetal tissues. *SEL-1L* is located on the long arm of chromosome 14 (14q24.3-31) in the region that corresponds to *IDDM11*, previously identified as a region showing evidence for linkage to IDDM. *SEL-1L* is composed of 21 exons spanning 70 kb of genomic DNA. In the present study we screened the 21 exons and the minimal promoter region (approximately 650 kb) for mutations by direct sequencing. Sequencing of 20 type 1 diabetic patients and 1 control did not reveal any mutations. However, mutations may still be found in the unanalysed part of the promoter region or in the introns of the gene.

A NOVEL MALIGNANT HYPERTHERMIA LOCUS SUGGESTED VIA LINKAGE SCREENING OF THE HUMAN GENOME. A. Olckers¹, D.A. Meyers², E. Taylor², D. Cawood¹, A. van der Merwe¹, Y. Havenga¹, G.S.

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Malignant hyperthermia (MH) is a complex autosomal dominant disorder that manifests as dysregulation of calcium ion metabolism in skeletal muscle. To date, six MH loci have been reported in the human genome: on 1q, 3q, 5p, 7q and 19q. A large South African MH family, MH102, does not display linkage to any of the reported MH loci. A core family of MH102 includes a total of sixteen individuals of whom six were previously phenotyped as MH susceptible (MHS) with the *in vitro* contracture test (IVCT), and three individuals as MH normal (MHN). The genome of the MH102 core family was screened with a set of short tandem repeat polymorphism (STRP) markers that span the genome with ca. 28 cM intervals. The highest maximum two point LOD score for family MH102 was calculated to be 2.716 ($q=0.001$) at the D2S72 locus, and is suggestive of linkage in this family. Only one other positive LOD score (1.188, $q=0.001$) was generated for this family. Sixteen individuals from this core family were genotyped with eight additional STRP markers. Recombination events in three MHS individuals and one obligate MHS individual excluded the D2S118 and D2S164 loci, respectively. The MHS(designated) haplotype was observed in five MHS individuals, one MHE individual and both of the obligate MHS individuals. Two of the MHN individuals did not inherit the MHS haplotype. This linkage data is suggestive of linkage in this large South African MH family to a novel MH locus on chromosome 2q.

Genetic Linkage of Bietti Crystallin Corneo-Retinal Dystrophy to Chromosome 4q35. *X. Jiao¹, J. Lee¹, F. Iwata¹, M. Hayakawa², A. Kanai², F.L. Munier³, D.F. Schorderet³, M.S. Chen⁴, M. Kaiser-Kupfer¹, J.F. Hejtmancik¹.* 1) Ophthalmic Genetics and Clinical Services Branch, National Eye Institute, Bethesda, MD; 2) Juntendo University, School of MEDICINE, Japan; 3) Oculogenetic Unit, Jules Gonin Eye Hospital and Division of Medical Genetics, Lausanne, Switzerland; 4) Department of Ophthalmology National Taiwan University Hospital, Taipei.

Bietti crystalline corneo-retinal dystrophy (BCD) is an autosomal recessive corneo-retinal degeneration characterized by multiple glistening intraretinal dots scattered over the fundus, degeneration of the retina, and sclerosis of the choroidal vessels, ultimately resulting in progressive night blindness and constriction of the visual field. Although BCD has been associated with abnormalities in fatty acid metabolism and the absence of fatty acid binding by two cytosolic proteins, the genetic basis of BCD is unknown. We carried out a genome-wide linkage screen for the BCD locus in 13 families of Chinese (2), Japanese (7), and European (4) ethnic origin. Two-point analysis gave evidence for linkage to markers on Chromosome 4q35 with maximum lod scores of 4.8 at $q = 0$ with D4S426 and 5.3 with marker D4S2299 at $q = 0$. Multipoint linkage analysis confirmed linkage to this region with a maximum multipoint lod score of 5.3 located 4 cM telomeric of marker D4S2930. Neither the M test for the three ethnic groups nor the admixture test (HOMOG) supported genetic heterogeneity. The linkage data strongly suggest that the BCD locus lies on Chromosome 4q35 near or telomeric to D4S2299. Studies currently to refine this interval and identify candidate genes.

5-Dye Implementation into ABI PRISM® Linkage Mapping Sets. *A. Wheaton, S. Wong, S. Abritz, R. Chhim, K. Upadhyaya, R. Brown, P. Hanachi, P. Dong.* Genetic Analysis R&D, PE Biosystems, Foster City, CA.

The incorporation of two new fluorescent dyes and a new size standard into our existing Linkage Mapping Sets (LMS) is described. Currently, our Linkage Mapping Sets are offered in four dyes: 6FAM™, HEX, NED™, and the ROX™-as a size standard label. We have expanded this current D dye set (Dye Set 30) to include a new dye, PET™, whose emission spectrum lies between NED™ and ROX™ at 592. In addition, we have replaced HEX with the spectrally similar VIC™ dye, which has both a higher peak intensity as well as a more favorable extinction coefficient than that of HEX. The new Linkage Mapping size standard is labeled with a new dye, which emits at a longer wavelength than ROX™ in the 5-dye chemistry. The introduction of the new 5-dye chemistry into LMS involves the replacement, as described above, of all HEX markers with the new VIC™ label; and the incorporation of PET™ into the existing schema. Two different configurations will be available: A 10-cM set will be available as a complete set, offering a mid-density option for lower resolution genome-wide screens. The 5-cM set, will be offered as a complete set, or by chromosome for higher resolution screens.

The addition of the new dyes will increase genotyping throughput significantly, as more markers can be simultaneously analyzed in a single lane or capillary. Use of the 5-dye system will increase the number of markers that can be run in a lane or capillary by as much as 33%. We display the performance of the 5-dye Linkage Mapping Set across the ABI PRISM® 377 DNA Sequencer, as well as the 310, 3700 and 3100 DNA Analyzers. We will demonstrate optimal spectral resolution, reliable signal intensity, and analysis tool compatibility with the new expanded 5-dye set.

MSS1 a mitochondrial GTP binding protein is involved in aminoglycoside sensitivity associated with deafness-linked mtDNA mutation. *M.X. Guan¹, J.Q. Mo², X.M. Li¹, X.L. Zhang¹, G. Faye³.* 1) Div Human Genetics, Children's Hosp Medical Ctr, Cincinnati, OH; 2) Dept. of Pathology, Univ. of Cincinnati College of Medicine, Cincinnati, Oh 45267; 3) Institut Curie, Section de Biologie, 91405 Orsay Cedex, France.

The use of aminoglycoside antibiotics causes a sensorineural hearing loss in genetically predispose individuals. The A to G transition at position 1555 (A1555G) in mitochondrial 12S rRNA has been found to be associated with aminoglycoside-induced deafness and non-syndromic deafness in human. Biochemical analysis showed that the A1555G mutation is the primary factor underlying the development of deafness, but the expression of the deafness phenotype needs the contributions of nuclear genes or aminoglycoside antibiotics. Recently, the corresponding mutation PR454 in mitochondrial 15S rRNA gene in yeast confers the resistance to aminoglycoside paromomycin. Previous studies have shown that mutant alleles of MSS1, encoding a mitochondrial GTP binding protein, manifest a respiratory-deficient phenotype only when coupled with the mitochondrial PR454 mutation. In the present work, the effect on mss1 mutations to aminoglycoside resistance has been analyzed in yeast strains derived from mss1 mutant and wild type carrying the PR454 mutation or lacking the mutation. Under the paromomycin-resistant background, in glycerol medium, mss1 mutations, particularly, in mss1-18 allele, can render the neomycin or paromomycin resistance resulted from the PR454 mutation. By contrast, in the paromomycin-sensitive background, mss1 mutations gave rise to the resistance to these drugs in glycerol medium. These results provided the first direct evidence that nuclear factor Mss1p is involved in mediating the aminoglycoside antibiotic toxicity associated with the PR454 mutation in mitochondrial 15S rRNA. These strongly indicate that Mss1p influences the phenotypic manifestation of the PR454 mutation, by interacting with the mitochondrial 15S rRNA at the decoding site, particularly at position of 1477. We proposed that the human homologue of MSS1 is a candidate gene influencing the expression of deafness phenotype associated with the A1555G mutation.

Analysis of the A1555G mtDNA mutation in Greek patients with sensorineural deafness. *E. Kleomitis¹, T. Iliadis², N. Voyiatzis², J. Economides³, P. Neou⁴, N. Apostolopoulos⁴, M. Grigoriadou¹, A. Pampanos¹, A. Skevas⁵, M.B. Petersen¹.* 1) Dept Genet, Inst Child Health, Athens, Greece; 2) Aristotle Univ Thessaloniki, Thessaloniki, Greece; 3) "Aghia Sophia" Children's Hosp, Athens, Greece; 4) "P & A Kyriakou" Children's Hosp, Athens, Greece; 5) Univ of Ioannina, Ioannina, Greece.

Many genes are known to be involved in deafness, located both in the nuclear and mitochondrial DNA. The homoplasmic A1555G mutation in the 12S rRNA gene of the mtDNA has been given a causative role for induction of progressive, non-syndromic, sensorineural deafness, which is enhanced by treatment with aminoglycosides. Previous studies have demonstrated a median age at onset of 5 years in those treated with aminoglycosides compared to a median age at onset of 20 years in those without treatment with these ototoxic drugs. The A1555G mutation has been detected in different populations regardless of genetic background and mtDNA haplotype. A recent study has shown a high proportion of this mutation in Spanish families with sensorineural deafness. In a collaboration with the major referral centers for childhood deafness in Greece, we examined 106 patients with sensorineural, non-syndromic deafness (101 prelingual, 5 postlingual with aminoglycoside exposure, all negative for the frequent 35delG GJB2 mutation). Patients were examined by PCR amplification and restriction enzyme digestion with HaeIII for the detection of the mtDNA A1555G mutation. The A1555G mutation was not detected in our sample. We conclude that the A1555G mutation is a rare cause of sensorineural, prelingual deafness in the Greek population. Possible explanations include that our prelingual patients were not ascertained exclusively due to aminoglycoside treatment or maternal inheritance, or a geographic difference in the prevalence of this mutation.

Mutation in cytochrome c oxidase subunit III in Alzheimer's brains. *F.J. Castora¹, N.S. Hambler², B. Conyers¹.* 1) Dept Physiological Sci, Eastern Virginia Medical Sch, Norfolk, VA; 2) School of Medicine, University California, San Diego, La Jolla, CA.

Mitochondrial dysfunction has become recognized as a critical component in the pathogenesis of a variety of neurodegenerative diseases, including Alzheimer's Disease (AD). Deficits in oxidative capacity, and specifically a reduction in cytochrome c oxidase activity have been reported in AD brains and platelets. We undertook an analysis of the three subunits of cytochrome c oxidase encoded on mitochondrial DNA (mtDNA) to detect AD associated mutations. We have identified a point mutation at np 9861 that occurs at a much higher frequency in AD brains than in age-matched controls. This mutation alters amino acid 219 of subunit III of cytochrome c oxidase from a phenylalanine to a leucine. This T9861C mutation appears to reduce cytochrome oxidase enzyme activity by almost 50% compared to wild type enzyme. The T9861C mutation is heteroplasmic and is not equally distributed throughout the brain. Functional characterization of this mutation is being pursued using the rho-zero cell system to generate oxidatively compromised cybrids.

Identification of novel paternally expressed transcripts from the Prader-Willi syndrome candidate region between *SNRPN* and *IPW*. Y-H. Jiang, Y-H. Huang, H. Li, K-S. Chen, A.L Beaudet. Dept Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Prader-Willi syndrome(PWS) is a neurobehavioral disorder characterized by neonatal hypotonia, childhood obesity, hyperphagia, mental retardation, and hypogonadism. Most PWS patients have either a paternally derived deletion of 15q11-q13 or maternal uniparental disomy. A 2 Mb region between *IPW* and *ZNF127* was defined from molecular characterization of a cryptic translocation within a PWS family, and paternally expressed transcripts(*SNRPN*, *PAR-1*, *PAR-5*, *IPW*, *ZNF127*, *NDN* and *NDNL*), and a biallelically expressed(*HERC2*) were identified. However, none of these genes are yet proven to be the gene responsible for the PWS phenotype. Several mutations have been introduced in the PWS homologous region in mice, and perinatal lethality was observed in all mice with paternally inherited mutations. Perinatal lethality was also observed in mice with a paternal deletion from *Snrpn* to *Ube3a* but not with a radiation-induced deletion extending from the *plocus* to *Ipw* which suggests that a structural gene or genes between *Snrpn* and *Ipw* contributes to the phenotype of mice with a *Snrpn-Ube3a* deletion. To identify novel human transcripts from the region between *SNRPN* and *IPW*, 4 BACs covering the region for which draft sequence has been deposited by the human genome sequencing project were identified by genome analysis using known genes and STS markers mapped to the region. The draft sequence of these BACs was re-edited manually, and then analyzed through database searching and by a variety of computational tools. Fourteen EST contigs from more than 100 ESTs were identified by these analyses. The high density of EST distribution in this region may suggest an unusual chromatin domain implicated in the imprinting process. The mapping position of these ESTs was confirmed by PCR and Southern using a PAC contig covering the region between *SNRPN* and *IPW*. Imprinting and expression analysis were done by RT-PCR and northern analysis. Nine paternally expressed human transcripts were identified, and several transcripts appear to be conserved in mice which suggests the functional significance of the transcripts.

Linkage studies suggest a possible locus for developmental dyslexia on chromosome 1p. *E.L. Grigorenko^{1,2}, F.B. Wood³, M.S. Meyer³, J.E.D. Pauls⁴, L.A. Hart⁵, D.L. Pauls¹.* 1) Psychology-Child Study Center, Yale University Sch of Med, New Haven, CT; 2) Dept of Psychology, Moscow State University, Moscow, Russia; 3) Section of Neuropsychology, Dept of Neurology, Bowman-Gray Sch of Med, Winston-Salem, NC; 4) Dept of Biochemistry, University of Vermont, Burlington, VT; 5) Dept of Psychology, University of Pittsburgh, PA.

Eight extended dyslexic families with at least four affected individuals (N = 165) were genotyped with twelve genetic markers spanning the Rh (rhesus factor) locus. Eleven of these markers were located on the short arm and the other was on the long arm of chromosome 1. Five theoretically derived phenotypes were used in the linkage analyses: (1) Phonemic Awareness; (2) Phonological Decoding; (3) Rapid Automatized Naming; (4) Single Word Reading; and (5) Vocabulary. In addition, a Lifetime diagnosis of dyslexia was used as a phenotype. Both parametric and nonparametric genetic analyses were completed. The results supported the importance of a putative locus on 1p, especially for the phenotypes of Single Word Reading and Phonological Decoding. In addition, two-locus analyses assuming the interaction between a 1p locus and a 6p locus, previously shown to be of interest for dyslexia, were conducted. The NPL scores for Rapid Automatized Naming and Phonological Decoding were significantly increased. In particular, the NPL scores for Rapid Automatized Naming exceeded 5.0 for certain markers. These results provide strong evidence for separate but jointly acting contributions of the 1p and 6p loci to the reading impairments associated with rapid naming and suggestive evidence for a similar mechanism involving phonological decoding.

Importance of differential methylation of a 12q13 specific RDA-derived retroviral related sequence (gb AF135486) in schizophrenia. *P. Deb-Rinker, R.L. O'Reilly, B.C. Murphy, S.M. Singh.* Molecular Genetics Unit, Western Science Centre, Univ Western Ontario, London, Ontario, Canada.

This report deals with the molecular characterization of a RDA-derived sequence (SZRV-2, GenBank Acc. # gb AF135486; Genome Database Acc. # GDB:7692183 and GDB:7501402) identified using DNA from monozygotic twin pairs discordant for schizophrenia. It represents a placentally expressed (~7 kb mRNA) endogenous retroviral-related (ERV) sequence. By fluorescence in situ hybridization we have mapped this primate specific sequence to 12q13, using two SZRV-2 positive BAC clones (4K11 and 501H16). The 12q13 is known to be the site for a number of viral-related sequences (potential hot spots for insertions), developmental, channel and signal transduction genes as well as genes affecting the expression of certain receptors in neurons. Of particular interest was the end sequencing of the 501H16 clone which showed sequence complementarity with two genes which have the potential to adversely affect neural development/function implicated in schizophrenia. Interestingly, this sequence is heavily methylated and may be subject to individual-specific epigenetic control. Further, aberrant methylation of the SZRV-2 genomic region in one of eight schizophrenia patients and none of the 21 unaffected controls, raises the possibility that methylation could play a role in this multi-factorial disease causing neuro-developmental abnormalities. It could account for non-genetic factor(s) required to account for the >50% discordance in MZ twins. Additional experiments currently underway should establish the significance of these results in schizophrenia.

Genotype-controlled analysis of plasma dopamine b-hydroxylase activity in unipolar psychotic depression. *J.F. Cubells^{1,2}, L.H. Price⁴, B. Meyers⁵, G.M. Anderson³, C.P. Zabetian^{1,2}, R.T. Malison², G. Alexopoulos⁵, G. Sanacora², J.C. Nelson², P. Kirwin^{1,2}, J. Gelernter^{1,2}.* 1) Psychiatry, Yale Univ Sch Med, New Haven, CT; 2) Psychiatry, VA Connecticut Health Care System, West Haven, CT; 3) Yale Child Study Center, New Haven, CT; 4) Psychiatry, Brown Univ Sch Med, Providence, RI; 5) Psychiatry, Cornell Univ Med Coll, White Plains, NY.

Dopamine b-hydroxylase (DbH) converts dopamine to norepinephrine. Plasma DbH level is a quantitative trait that maps to the DBH locus. Patients with psychotic depression (PDEPs) exhibit lower plasma DbH levels than nonpsychotic depressives (NDEPs). To test the hypothesis that genetic variation at DBH influences vulnerability to psychotic features in depression, we measured plasma DbH levels in healthy European-Americans (EA; N = 29), EA PDEPs (N = 23), and EA NDEPs (N = 32), and genotyped DBH*444g/a, a synonymous single nucleotide polymorphism at DBH associated with variation in plasma DbH levels. ANOVA showed significant effects of DBH genotype ($p = 0.003$) and diagnosis ($p < 0.001$) on plasma DbH activity; age differed significantly among groups (PDEPs were the oldest), but covarying for age in the analysis did not change the results. As in previous studies, PDEPs exhibited significantly lower plasma DbH activity than NDEPs ($p < 0.05$) or healthy EAs ($p < 0.01$), and the DBH*444a allele associated with lower DbH activity than the DBH*444g allele. Contrary to our hypothesis, there was neither a significant genotype-diagnosis interaction, nor significantly different DBH*444g/a allele or genotype frequencies among the diagnostic groups. The results suggest two possibilities: (1) a variant(s) other than that examined here, at DBH or at another locus, lower(s) plasma DbH activity and increase(s) risk for PDEP; or (2) lower plasma DbH reflects altered neurobiological (possibly noradrenergic) function associated with PDEP. Supported by NARSAD, USDVA, NIDA, NIMH, and NIAAA.

LACK OF ASSOCIATION BETWEEN A SEROTONIN TRANSPORTER PROMOTER POLYMORPHISM AND BIPOLAR DISORDER IN ANTIOQUIA, COLOMBIA.

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We have examined the frequency of a functional length polymorphism in the promoter region of the serotonin transporter (5-HTTLPR) in a case/control sample for bipolar mood disorder type 1. Patients (N=98) and controls (N=104) were collected from the isolated population of Antioquia, Colombia. Cases were evaluated using the Diagnostic Interview for Genetic Studies (NIMH). Normal controls were randomly selected individuals matched with cases for sex and over 25 years old. Genomic DNA samples were genotyped using the experimental conditions reported by Lesch et al. (Science 274, 1527-1531). Allele frequencies for the cases were $l = 0.52$ $s = 0.48$ and for the controls $l = 0.47$ $s = 0.53$. Genotype frequencies for the cases were $l/l = 0.29$ $l/s = 0.45$ $s/s = 0.26$ and for the controls: $l/l = 0.19$ $l/s = 0.56$ $s/s = 0.25$. No statistically significant deviation from Hardy Weinberg expectation was detected in the two groups. No statistically significant difference in the allele (chi square 0.47, $p=0.493$) or genotype (chi square 3.08, $p=0.214$) frequencies was observed between cases and controls, thus we detected no evidence of association of this polymorphism with bipolar disorder in this sample. This work was supported by Universidad de Antioquia (CODI grant #9845), Colciencias (grant #1115-04-414-98) and the Wellcome Trust (grant #056081).

SNP analysis of GABA_A receptor subunit in autistic disorder. *M.M. Menold¹, M.P. Bass¹, J.R. Gilbert¹, C.M.*

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Gamma-aminobutyric Acid (GABA) is the major inhibitory neurotransmitter in the adult brain acting via the GABA_A receptors. GABA_A receptors are comprised of several different homologous subunits forming a group of receptors that are both structurally and functionally diverse. The GABA_A- β 3, - α 5, and - γ 3 subunit genes form a cluster on chromosome 15q11-q13, in the region implicated to be harboring genetic risk factors for Autistic Disorder (AD) (Bass et al., 1999). In particular, linkage disequilibrium (LD) has been reported with microsatellite markers in GABRB3 (Cook et al., 1998; Martin et al., 1999). The LD was strongest in our data when examined in the subset of nuclear families (Martin et al., 1999).

To further investigate the relationship between the GABA_A receptors and AD, we genotyped three GABRB3 single nucleotide polymorphisms (SNPs) in our data set of 91 multiple incidence (79 nuclear families; 12 extended pedigrees) and 124 single incidence AD families. Genotyping was performed using either OLA (oligonucleotide ligation assay) or SSCP (single strand conformation polymorphism) followed by DNA sequencing. We tested for LD in the data for each SNP independently using the Pedigree Disequilibrium Test (PDT) as well as in the GABRB3 SNP haplotype data using Transmit. Analysis of the entire data set showed no evidence for LD (PDT: GABRB3-30C/G p=0.37; GABRB3-170C/T p=0.59; GABRB3-1427C/T p=0.60; Transmit: p=0.67). In the subset of 79 nuclear families, we found suggestive evidence for LD in GABRB3-1427C/T (p=0.08) and in the haplotype data (p=0.08). Typing of GABRA5 SNPs in the same region is ongoing and will be included in the analyses.

Challenges of two rare syndromes affecting the nervous system in members of one large kindred. *D.E. McNeil¹, W.M. Linehan², G.M. Glenn¹*. 1) Genetic Epidemiology Branch, DCEG, National Cancer Institute, Rockville, MD; 2) Urologic Oncology Branch, DCS, National Cancer Institute, Bethesda, MD.

We identified a family with two autosomal dominant inherited conditions affecting the nervous system: Von Hippel-Lindau disease (3p25-26); and Spinocerebellar Ataxia, type 2 (12q24), also called olivopontocerebellar ataxia (OPCA). Initially the family diagnosis of VHL was known, but the genetic basis of the ataxia was unknown. Our aim was to find the exact gene and the mutation responsible for the ataxia. We also performed clinical screening tests to determine which condition was responsible for the patients' neurologic deficits, in order to identify any treatable lesions. Spinocerebellar Ataxia, type 2 (SCA2) is a neurodegenerative disease characterized by progressive cerebellar signs, pyramidal signs and, in some individuals, mild dementia. Von Hippel-Lindau disease (VHL) is a neoplastic disorder predisposing to specific tumors of the central nervous system, inner ear, eye, kidney, pancreas, and adrenal glands. The earliest known affected member of this kindred was a female with both conditions. Of her sixteen children, five (two males, three females) had OPCA and four (one male, three females) had VHL. Her eldest child (a male) had both conditions. Among her grandchildren five had OPCA (three males and two females) while five had VHL (two males, three females). A female grandchild has both conditions with onset in her teens of retinal and visceral VHL symptoms, and in her late twenties she developed progressive neurologic symptoms. On MRI, she had no cerebellar VHL hemangioblastomas to explain her symptoms, but cerebellar atrophy was seen. Genetic analysis of some family members revealed a unique point mutation in the VHL tumor suppressor gene. In addition, the female with both conditions had an abnormal increase in the number of trinucleotide repeats of her SCA type 2 gene.

Genetic association between Alzheimer disease and alpha-synuclein gene. *M. Matsubara*¹, *H. Yamagata*^{1,2}, *K. Kamino*³, *T. Nomura*¹, *K. Kohara*¹, *I. Kondo*², *T. Miki*¹. 1) Dept Geriatric Medicine, Ehime Univ Sch Medicine, Ehime, Japan; 2) Dept Hygiene, Ehime Univ Sch Medicine, Ehime, Japan; 3) Dept Geriatric Medicine, Kanazawa Medical Univ, Ishikawa, Japan.

NACP/alpha-synuclein has been isolated as a component of amyloid in addition to the major A-beta peptide in Alzheimer disease. However the pathological pathway in AD has not been determined. During screening for the alpha-synuclein gene mutation using DGGE and subsequent sequence analysis, we detected a novel and common polymorphism in intron 3. This polymorphism could easily be detected by PCR-RFLP method using BsrI, where a 2 bp (CT) deletion (D) and insertion (I), with respective frequencies of 0.41 and 0.59, were observed in our Japanese control population. To resolve this issue, we examined the relationship between AD and alpha-synuclein and ApoE genes in 183 Japanese AD patients and 210 controls. Carriers of the deletion allele had 2.2-fold increased risk for developing AD than non-carriers in women. The results showed that the alpha-synuclein gene is associated with sporadic AD in women, independent of ApoE4 status.

Cretan families with late onset HD associated with stable CAG expansion share a common haplotype. E.K.

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We have previously reported (Hum. Mol. Genet. 4:2239, 1995) that a unique form of Huntington's disease (HD) characterized by onset in late life (5th-8th decade) and absence of anticipation occurs on the island of Crete. In affected families, the expanded HD CAG repeat is passed stably during meiotic transmission. Here, we constructed a haplotype of 8 polymorphic markers (D4S43, D4S136, D4S182, D4S95, D4S127, D2642, D4S126, ADRA2C) flanking the HD locus and spanning a 1.5 cM region (in 4p16.3) in eight families with late onset HD and in one family with the typical form of this disorder of Cretan origin. Five out of the eight late-onset HD families originate from a single village located in the Lasithion county, while the remaining three originate from three different villages located in the Heraklion county. The typical HD family originates from the Rethymnon county. Results showed that all five late-onset HD families originating from a single village share an extended, disease-associated haplotype of 1.5 cM between D4S43 and ADRA2C. The highly conserved region shared by all mutant chromosomes (N=37) of individuals from all eight late-onset HD families (originating from four different villages) is defined by the polymorphic markers D4S95, D4S127, D2642, which are located <0.5 cM apart. Marker D2642 is intragenic, whereas markers D4S95 and D4S127 are within 0.5 cM from the HD gene. In contrast, patients belonging to the family with typical HD showed a distinct haplotype, including the intragenic marker D2642. Sequencing of exon 1 of the IT-15 gene containing the (CAG)_n repeat of patients with late-onset HD revealed that the expanded repeat did not contain any interruption, which could account for the stability of this DNA segment during meiotic transmission. These data favor the independent origin of late-onset and typical HD in this population. In addition, they provide strong evidence that all late-onset HD cases relate to a common ancestor, and that the part of the HD chromosome containing the markers D4S95, D4S127 and D2642 is crucial for the stability of the (CAG) repeat.

Human homolog of the mouse imprinted gene *Impact* resides at pericentric region of chromosome 18 within the critical region for bipolar affective disorder. *R. Kosaki*¹, *T. Suzuki*², *H. Yoshihashi*², *Y. Goto*³, *N. Matsuo*², *K.*

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Several mapping studies of families with multiple individuals who have bipolar affective disorder (BPAD) have demonstrated possible linkage of the trait to the pericentric region of chromosome 18 (18cen). Currently, the large size of the critical interval defined by these studies makes effective selection of candidate genes formidable. However, documentation 18cen-linked families in which parent-of-origin effect was observed in the transmission of the BPAD trait provides a clue as of the nature of the putative gene: It may be imprinted. In the present study, we cloned *IMPACT*, the human homolog of the mouse imprinted gene *Impact* and mapped it to 18cen within the critical interval for BPAD and demonstrated that human *IMPACT* is expressed highly in the brain. Because small number of imprinted gene are estimated to be present in the entire genome, only a few imprinted genes are expected to be present at a particular chromosomal region. Hence, *IMPACT* represents a candidate gene for BPAD susceptibility. Alternatively, other yet unknown imprinted gene(s) neighboring *IMPACT*, if any, could contribute to the BPAD trait, considering that multiple imprinted genes, in some cases, exist in a confined interval forming a cluster. Cloning and localization of human *IMPACT* at 18cen in this study defines a promising target region where putative BPAD genes could be sought for.

Association between the SCN1B gene and idiopathic generalized epilepsy. *K. Haug¹, J.S. Dullinger¹, K. Hallmann¹, B. Rau¹, S. Beyenburg², P. Propping¹, C.E. Elger², A. Heils^{1,2}.* 1) Inst Human Genetics, Univ of Bonn, Bonn, Germany; 2) University Clinic of Epileptology, Bonn, Germany.

A missense mutation in the gene encoding the 1-subunit of neuronal sodium channels (SCN1B) has recently been shown to cause epilepsy in a family with generalized epilepsy with febrile seizures plus (GEFS+). The present study was designed to test the hypothesis that variation in the SCN1B gene confers increased liability to common subtypes of idiopathic generalized epilepsy (IGE) including juvenile myoclonic epilepsy (JME) and childhood absence epilepsy (CAE). Length variation of an SCN1B intragenic polymorphic trinucleotide repeat marker (TTA)_n were assessed in a sample consisting of 108 IGE nuclear families ascertained for an affected child. A bi-allelic transmission disequilibrium test (TDT) considering the two most frequent alleles as well as a multi-allelic TDT based on the procedures introduced by Spielman and Ewens [Am J Hum Genet, 1996, 59: 983-989:] were performed. 6 different alleles were detected in our sample, of which the 8-copy and 11-copy alleles accounted for more than 75% of all alleles. In 112 cases, the alleles transmitted and non-transmitted could unequivocally be assigned from heterozygous parents. The 8-copy allele was transmitted 10 out of 48 times, whereas the 11-copy allele was transmitted 79 out of 127 times. From the bi-allelic TDT there is strong evidence that the 8-copy allele is protective with an asymptotic P value of 0.000053. Furthermore, there is suggestive evidence that the 11-copy allele is associated with the disease (corresponding P value 0.00595). In the multi-allelic TDT, a $T_{\text{mhet}} = 24.91$ was found, which corresponds to an asymptotic P value of 0.0000524. Our study provides strong evidence that the SCN1B gene is associated with common IGE subtypes including JME and CAE. Thus, a systematic search for mutations in the SCN1B coding region and the regulatory sequences is in progress in our laboratory.

Spinocerebellar ataxia 7 (SCA7): Identification and characterization of a new partner for ataxin-7. A.S. Lebre¹, L. Jamot¹, J. Takahashi², N. Spassky³, P. Kussel⁴, C. Duyckaerts², J.H. Camonis⁵, A. Brice¹. 1) INSERM U289, Hopital de la Salpetriere, Paris, France; 2) Laboratoire de Neuropathologie, Hopital de la Salpetriere, Paris, France; 3) INSERM U495, Hopital de la Salpetriere, Paris, France; 4) Institut Pasteur, Paris, France; 5) INSERM U528, Institut Curie, Paris, France.

Spinocerebellar ataxia 7 (SCA7) is a progressive neurodegenerative disease associated with degeneration of optic pathways and the retina and caused by the expansion of a CAG repeat in the coding region of the gene. Ataxin-7 is a protein of unknown function. The mutated protein has been reported to be nuclear, as analyzed by Western blot on cell fractions of lymphoblasts from SCA7 patients. In contrast, expression of ataxin-7 is cytoplasmic and nuclear in normal human brain. Despite the ubiquitous expression of ataxin-7, mutations lead to the death of only certain neuronal populations. The phenotype might be explained by an interaction with a partner that is specific to the vulnerable cells. Using a two-hybrid approach, we screened a human neural retina cDNA library for ataxin-7 binding proteins. We isolated 21 different clones that interact with normal and mutated ataxin-7. We investigated the role of one of these proteins, CAP (Cbl Associated Protein) or ponsin, previously described as a Cbl partner. We isolated a splice variant of CAP with a nuclear localization signal. This protein has been selected for further studies on the basis of its expression in the CNS. The interaction between ataxin-7 and CAP was confirmed by GST pull-down. CAP co-localizes in the nucleus of COS-7 and HEK293 co-transfected cells with wild-type ataxin-7 and is sequestered in nuclear inclusions when co-transfected with mutated ataxin-7. Endogenous CAP is expressed in the nucleus and the cytoplasm of neurons in primary neuronal cultures (cerebellum and mesencephalon) as well as in normal human brain. We confirmed that CAP is localized within the nuclear inclusions in SCA7 brain. More investigations are underway to further characterize the role of CAP in the normal function of ataxin-7 and how it could be involved in pathophysiology of SCA7.

Expression array profile based identification of candidate genes for complex response phenotypes with specific reference to alcohol. *B.C. Murphy, M.L. Harrison, R.K. Uddin, T. Chiu, J.A. Treadwell, S.M. Singh.* Dept Molecular Genetics, Univ Western Ontario, London, ON., Canada.

Individual specific responses to a number of addictive drugs and chemicals are recognized to have a genetic basis. Attempts to identify such genes using traditional methods have been hampered by the complexities of genetic heterogeneity and environmental causation. The addictive effect of these drugs and chemicals is attributed to their direct or indirect effect on expression for a specific set of genes. This now offers the prospect of identifying these genes using expression arrays. Viewed in the context of families or suitable animal models, they have the potential to serve as candidate genes. In this ongoing project we have assessed the feasibility of such an approach in the context of alcohol effect on genetic strains of mice, with variable preferences for ethanol using cDNA expression arrays from Clontech. Brain and liver RNA was isolated from ethanol treated (E) and matched control (C) mice from four strains. The cDNA from C57BL/6J (alcohol preferrer) and A/J, BALB/cJ, DBA/2J (alcohol avoiders) was used for hybridization to array membranes. The resulting autoradiographs identified that most brain and liver specific genes are not affected by acute or chronic ethanol treatment. Some of the affected genes (e.g. NADH cytochrome P450 reductase, IGFBP-1, I-kB alpha) were up-regulated in all strains tested whereas other genes (e.g. Rac 1 murine homologue and Syp:SH-PTP2) were strain-specific in their response to ethanol. With few exceptions, the effect of ethanol was found to be subtle (i.e. difference was less than 2X) but reproducible in repeated hybridizations. This may suggest that the variable effects of alcohol and other addictive drugs may be due to subtle changes in a number of genes, that can be reliably identified by the increasing sophistication of the expression array technology. Identification of such genes is important for our understanding of the physiological basis and addictive mechanisms of alcoholism. (Supported by MRC and OMHF).

Genetic analysis of the D4DR, DAT1, and DbH genes in Sib Pairs with ADHD. *B.A. Navia^{1,2}, K.M. Smith², Y. Kleyner², M.J. Daly³, E. Lander³, R. Barkley⁴.* 1) Neurol, Gen/NE Medical Ctr, Tufts Univ Sch Medicine, Boston, MA; 2) Gen Prog/ Sackler School GBS, Tufts University, Boston, MA; 3) Whitehead Institute, MIT, Cambridge, MA; 4) Dept of Psychiatry, UMass Medical Center, Worcester, MA.

Attention Deficit Hyperactivity Disorder (ADHD) is a common and highly heritable disorder with three subtypes: Inattentive (I), Hyperactive/Impulsive (H), and Combined (C) types. Several groups have shown a significant association with polymorphisms in the D4 Dopamine Receptor (D4DR) gene, the Dopamine Transporter (DAT1) gene, and the Dopamine b Hydroxylase gene, suggesting that alterations in dopaminergic signaling or metabolism contribute partly to ADHD. We have performed a genetic study of affected sib pairs to determine whether the different subtypes of ADHD show preferential transmission of these candidate susceptibility alleles. Thirty-five affected sibling pairs and their parents have been genotyped for the D4DR exon 3 VNTR, the DAT1 3'UTR VNTR, and the DbH polymorphic GT repeat. TDT was performed to test for preferential transmission of the D4DR 7 repeat allele, the DAT1 480 bp allele, and the DbH A4 "high activity" allele.

The analyzed sib pairs included: 14 families with I/C pairs, 9 with I/I pairs, 8 with C/C pairs, 3 with I/H pairs, and 1 family with a C/H pair. Combining the genotype data from the I/C, C/C, I/H, and C/H families, the frequency of the D4DR 7 repeat allele was 3 times higher in this group compared to the frequency of this allele in the pure Inattentive or I/I families (.179 vs. .056). Similar analysis of the DAT1 and DbH polymorphisms revealed no such differences. Preferential transmissions of the D4DR 7 repeat allele, the DAT1 480 bp allele, or the DbH A4 allele were not observed by TDT. However, a trend towards increased transmission of the DbH A4 allele was observed by TDT in I/I families ($p=.157$). These results support the hypothesis that the I/C and C/C families share common genetic factors and that the pure inattentive form of ADHD (I/I families) may represent a genetically distinct disorder.

Genetic study of generalized and partial epilepsy - linkage on chromosome 5. *M.Z. Labuda, A. Siren, S. Bourgon, J.M. St-Hilaire, K. Simola, M. Koivikko, E. Andermann, M. Pandolfo.* Ctr de Recherche , CHUM, Montreal, Quebec, Canada.

We have studied 7 families with dominantly inherited idiopathic epilepsy. Two French Canadian, one American, and one Italian family segregate temporal lobe epilepsy (TLE) with simple or complex seizures. In these families patients have a vegetative aura or déjà vu symptoms. Two Finnish families segregate febrile convulsion (FC). TLE families had been previously tested for known partial epilepsy loci on chromosomes 20q, 15q, 10q, 8q, 19q, 22q, whereas Finnish families had been tested for febrile convulsion loci on chromosomes 2q, 19p, 19q and 11. All candidate loci were excluded. Subsequently, a genome-wide scan was started. We have identified a marker on chromosome 5 co-segregating with the epileptic phenotype in 3 families. One of these families is French Canadian, the phenotype consisting in temporal, fronto-temporal, or occipital epilepsy. Two families are Finnish and most affected subjects have FC and/or childhood absence epilepsy. However, in one of these families, two siblings have déjà vu (symptom of temporal epilepsy) and their first cousin has déjà vu and FC. One of the subjects with déjà vu has a child with FC, the other has one child with idiopathic generalized epilepsy and one with FC. The cumulative lod score for marker D5S2500 in these 3 families was 3.0 at $\theta=0.05$. We are investigating additional markers in the region of interest. Our results suggest that mutation(s) in the same gene might cause partial as well as generalized epilepsy.

Genetic characterization of Spanish dominant ataxia cases. V. Volpini¹, J. Corral¹, I. Banchs¹, O. Combarros², D. Genis³, J. Berciano². 1) Molecular Genetics Dept, Cancer Research Institute, Barcelona, Spain; 2) Neurology Service, Hosp Valdecillas, Santander, Spain; 3) Neurology Unit, Hosp J Trueta, Girona, Spain.

Autosomal dominant cerebellar ataxias (ADCA) have been classified phenotypically as ADCAI, characterized by ataxia and "plus" neurological signs; ADCAII which adds the anterior signs retinal degeneration and ADCAIII, group of "pure" ataxia. ADCAI comprises the loci: SCA1 (6p22), SCA2 (12q23), SCA3 (14q32.1), SCA4 (16q22.1, uncloned gene), SCA8 (13q21) and SCA12; ADCAII: SCA7 (3p12); ADCAIII: SCA5 (11p12, uncloned), SCA6 (19p13), SCA10 (22q13, uncloned) and SCA11 (15q14, uncloned). All but two genes cloned show a CAG repeat expansion, which encodes a polyglutamine tract. The exceptions are SCA12, in which the CAG is in the 5' untranscribed region; and SCA8, which consists in an exonic but untranslated CTG repeat. We have detected the mutations in 46 of 111 unrelated familial Spanish cases of ADCA (41.4%) and in none of 157 sporadic cases. For familial ADCA cases with mutation 7 (15.2%) were SCA1; 14 (30.4%) SCA2; 19 (41.3%) SCA3; 2 (4.35%) SCA6; 4 (8.7%) SCA7 and none SCA8 or SCA12. The expanded alleles ranged from 41 to 59 for SCA1; 35 to 53 for SCA2; 58 to 79 for SCA3; 23 to 25 for SCA6; and 38 to 113 for SCA7. The CAG expansion increases through generations mainly in male transmissions. Meiotic segregation analysis does not show preferential transmission of the expanded allele. We also presented a family in which we have not detected any of the reported mutations. In addition, genetic linkage analysis shows that the locus trait of this family is unlinked to all the previously reported SCA loci. Linkage simulation analysis with 1000 replicates gives a power or $\text{Prob}(Z_{\max}(r) > 3) = 21\%$ and an expected lod score $E[Z(q=r)] = 1.72$, at "true" recombination fraction value: $q = r = 0.05$.

HOPA polymorphisms are associated with an increased risk for hypothyroidism and psychosis. *R.A. Philibert, H.K. Sandhu, A.M. Hutton, Z. Wang, S. Arndt, N.C. Andreasen, R. Crowe, T. Wassink.* Psychiatry, Psychiatry Research/MEB, Iowa City, IA.

HOPA is an Xq13 chromosome gene that codes for a thyroid receptor (RXR nuclear receptor) transcriptional co-activator. In a prior study of the genetic basis of schizophrenia, we demonstrated that exonic polymorphisms in HOPA were associated with increased risk of schizophrenia and hypothyroidism in a large cohort of probands from New York. In an attempt to replicate these findings, we examined this relationship in a cohort of 173 schizophrenic patients (128 males and 45 females providing 218 alleles) from Iowa. Consistent with the prior findings, we found an increased rate of the HOPA12bp exonic HOPA polymorphism in schizophrenic probands as compared to random newborn controls (9 of 218 alleles vs 33 of 2049 alleles, $p < 0.02$). Furthermore, retrospective review of the medical records demonstrated that two of the nine probands possessing the HOPA12bp allele in whom thyroid function was assessed were hypothyroid as compared to 6 of 164 probands possessing the normal HOPAwild allele(s) ($p < 0.06$). We conclude that the HOPA12bp polymorphism may be a vulnerability factor for both schizophrenia and hypothyroidism and that further studies are required to define the features of this syndrome and the molecular mechanisms of disease pathogenesis.

The Apolipoprotein E Gene Modulates Risk for Familial Age-Related Macular Degeneration. *S. Schmidt¹, R. Heinis¹, A.M. Saunders¹, M. De La Paz¹, E. Postel¹, W.K. Scott¹, T. Kelley², A. Agarwal², A. Bazyk², J.L. Haines², M.A. Pericak-Vance¹.* 1) Duke University Medical Center, Durham, NC; 2) Vanderbilt University Medical Center, Nashville, TN.

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss in older adults, affecting at least 15 million people in the United States. Previously, Souied et al. (1998) and Klaver et al. (1998) noted that the e4 allele of the apolipoprotein E gene (APOE) has a protective effect on the risk of AMD and that the e2 allele increases risk. We examined the role of APOE in our independent AMD data set. Our data include the largest number of cases studied so far (n=231), of which n=130 are familial and n=101 sporadic. Controls (n=240) were n=201 spouses of Alzheimer disease patients and n=39 spouses of AMD patients examined for AMD and found to be unaffected. APOE genotype and allele frequencies were compared between cases and controls by use of χ^2 statistics and multiple logistic regression analysis. Significant age and sex differences between the case and control group were found ($p < 0.001$). The age- and sex-adjusted odds ratio (OR) from the logistic regression model comparing e4 carriers with the e3/e3 genotype was 0.75 (95% CI (0.47, 1.18), $p = 0.21$). Analysis stratified by family history of the cases detected a significant protective effect of the APOE e4 allele for the n=130 familial cases, where the crude OR for e4 carriers compared to the e3/e3 genotype was 0.50 ((0.29, 0.86), left-sided $p = 0.007$). The age- and sex-adjusted OR from the logistic regression model for familial cases was 0.55 ((0.31, 0.96), $p = 0.04$). While the overall APOE genotype distribution among familial cases was not significantly different from that among controls ($p = 0.09$), the allelic distribution was ($p = 0.02$). No significant differences with respect to APOE genotype or allele frequency were found in the comparison of n=101 sporadic cases with n=240 controls. Our power to detect an effect of the rarer e2 allele was limited. This study supports the protective effect of the APOE e4 allele on the risk of familial AMD and suggests an underlying biological role of APOE in AMD etiology.

No Apolipoprotein E genotypes effect on the age at onset of Parkinson's disease. A. Parsian¹, B. Racette², L.J. Goldsmith¹, D. Miles¹, M. Rundle², J.S. Perlmutter². 1) Birth Defects Center and Dept of Fam and Com Medicine, Univ Louisville Health Sci Ctr, Louisville, KY; 2) Dept. of Neurology, Washington Univ Med Sch, St. Louis, MO.

Idiopathic Parkinson's disease (PD) is an age dependent, neurodegenerative condition frequently associated with dementia. Although it is predominantly a sporadic disease, 20-30% of cases are familial suggesting a complex mode of inheritance. Apolipoprotein E (ApoE) allele e4 has been associated with familial and sporadic late onset senile dementia of the Alzheimer's type. It is reported that ApoE genotypes modulate the age at onset of PD and the patients with e4 allele have the earliest onset (Zareparsian et al., 1997). To investigate the role of ApoE in susceptibility to PD, specially the age at onset, and dementia associated with PD, we screened a sample of PD patients with (n=118) and without (n=166) a family history, as well as matched normal controls (n=94). Kaplan-Meier survival analysis was used to plot genotype-specific age at onset distribution curves. The differences between the curves were also tested using log-rank statistics. Allele and genotype frequencies of the ApoE gene in PD patients with and without a family history and normal controls were not significantly different. However, the frequencies of e4 allele and e3/e4 genotype in the PD group with dementia were two fold higher than in normal controls but the differences were not significant. The familial PD had significantly earlier age at onset than sporadic PD (log-rank test, p=0.027). The age at onset distribution curves for e4/-, e3/e3, and e2/e3 genotypes were similar and their differences were not significant (p=0.38). We conclude that ApoE gene does not play an important role in susceptibility to PD nor it modulates the age at onset of PD.

Genetic analysis of onset age of Parkinson's disease: Apolipoprotein E. *S. Zareparsil¹, J. Kaye^{1,2}, R. Camicioli¹, S. Ganther³, J. Nutt¹, T. Bird^{4,5}, H. Payami¹*. 1) Dept Neurology, Oregon Health Sciences University, Portland, OR; 2) Portland Veterans Affairs Medical Center, OR; 3) Kaiser Permanente, Portland OR; 4) Veterans Affairs Puget Sound Health Care System, Seattle, WA; 5) Dept Neurology, University of Washington, Seattle, WA.

Parkinson's disease (PD) has a variable age at onset that can span several decades. It has been suggested that the variation in age at onset of PD may be influenced by familial factors including genes. We studied the effect of the apolipoprotein E (APOE) gene on onset age of PD because a) PD exhibits clinical and neuropathological overlap with Alzheimer's disease (AD), b) APOE-e4 allele is associated with increased risk and earlier onset of AD, and c) we had previously observed that e4 was associated with earlier onset of PD in a preliminary study of 137 patients. We collected 460 new unrelated Caucasian PD patients to provide sufficient statistical power to confirm or refute the original observation. The Z statistic and the Student's t-test were used to compare allele frequencies and mean onsets respectively. The APOE-e4 allele was associated with a significant 3-years earlier onset of PD ($p=0.01$). Classification of subjects into early-onset (onset<50 years) and late-onset PD revealed interesting results. In early-onset PD, there was no detectable e4 effect on age at onset, but e4 allele frequency was significantly increased compared to late-onset PD ($p<0.025$). In late-onset PD, e4 was associated with a significant 2-years earlier onset of PD ($p=0.03$), but e4 allele frequency was similar to the general population. The results demonstrate that APOE is associated with PD, however it is not currently clear if the association is with age at onset or with susceptibility to early-onset PD.

Asp90Ala (A90D) mutation and novel rare normal polymorphism IVS3+35 A>C in Cu-Zn superoxide dismutase gene in sporadic amyotrophic lateral sclerosis patients from Russia. *P.A. Slominsky¹, M.I. Shadrina¹, E.A.*

Kondratyeva¹, T.V. Tupitsina¹, G.N. Levitsky², V.I. Skvortsova², S.A. Limborska¹. 1) Dept Human Molec Genetics, Inst Molecular Genetics, Moscow, Russia; 2) Dept of Neurology, Russian State Medical University, Moscow, Russia.

Amyotrophic lateral sclerosis (ALS) is degenerative disorder of motor neurons in cortex, brainstem and spinal cord. It is now recognized that 10-20% of FALS cases are associated with mutations in the gene encoding the free radical scavenging enzyme CuZn-superoxide dismutase (CuZn-SOD1). The mutations have all shown autosomal dominant trait except for A90D, which may also be recessive. We observed 21 patients within age range 29-73 years with idiopathic motor neuron disease. We have identified the missense point mutation Asp90Ala in two our patients. In the first case, patient, 67-year-old female, have a typical case of adult-onset autosomal recessive ALS. We found similar heterozygous SOD1-mutation in her daughter (36-year-old) with no signs of motor neuron disease This patient is homozygous for the D90A mutation. In the second case, patient, 57-year-old female, have ALS case with lumbar onset and rapid progression. She is heterozygous for Asp90Ala mutation. Mutation D90A was also found in one health person Russian descendent from Novgorod district (north-west part of Russia) and not observed in random sample from two other Russian populations - from Kursk and Arkchangel'sk districts. Besides, we identified new intronic point mutation IVS3 + 35A>C in two typical sporadic ALS patients. This nucleotide transition introduces a new site for restriction enzyme HspA1. However, IVS3+35 A>C polymorphism was observed in random sample from three populations from Russia (Russians from Kursk and Arkhangelsk regions and Yakuts). The low frequency of this mutation (0.017- 0.03) was observed. So, this intronic mutation is rare normal polymorphism - not ALS causative mutation.

Molecular diagnosis of spinocerebellar ataxias in a Mexican population. *A. Rasmussen¹, P. Yescas¹, T. Matsuura^{2,3}, T. Ashizawa^{2,3}, ME. Alonso¹.* 1) Department of Neurogenetics, Instituto Nacional de Neurologia y Neurocirugia, Mexico City, Mexico; 2) Department of Neurology, Baylor Medical College, Houston, Texas; 3) Department of Neurology, Veterans Affairs Medical Center, Houston, Texas.

Autosomal dominant spinocerebellar ataxias (ADCA) are a clinically and genetically heterogeneous group of neurodegenerative disorders caused by expansions in unstable microsatellite repeats in the SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA10, SCA12 and DRPLA genes.

We present the mutation analysis of the SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA10, SCA12 and DRPLA genes in 62 Mexican individuals with ataxia: 36 unrelated familial (28 autosomal dominant, 7 autosomal recessive) and 26 sporadic cases. For ADCA cases, we found 12/28 (42.85%) SCA2, 4/28 (14.28%) SCA10, 3/28 (10.71%) SCA7 and 2/28 (7.1%) SCA3 mutations. No mutations were detected in the remaining genes. As expected no SCA or DRPLA mutations were found in the suspected autosomal recessive kindreds, but one sporadic case showed an abnormal 44 repeat allele of the SCA2 gene. 25% of ADCA cases remain undiagnosed.

The distribution of mutations in the SCA genes is different from what is usually reported, our population has more than 50% of the ADCA cases related to mutations in only two genes: SCA2 and SCA10. Matsuura et al. have found a founder effect underlying the high SCA10 prevalence in Mexican population, such an effect should also be analyzed regarding SCA2.

The clinical overlap among the different genetic entities makes prediction of the molecular origin in a single patient impossible, so that molecular characterization is necessary.

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Genomic analysis of the breakpoints in balanced t(9;11)(p24;q23.1) translocation that partially co-segregates with bipolar affective disorder in a small family. *J.E. Willett-Brozick, B.E. Baysal.* Psychiatry, Univ. of Pittsburgh Med. Ctr., Pittsburgh, PA.

Bipolar affective disorder (BAD) is a complex life-threatening disorder characterized by pathological mood disturbances ranging from mania to severe depression. Family, adoption and twin studies suggest a considerable genetic contribution to its pathogenesis. A small family showing co-segregation of a balanced chromosomal translocation t(9;11)(p24;q23.1) with BAD was previously described. We hypothesized that a disrupted gene(s) may have contributed to the disease pathogenesis. We previously established somatic cell hybrids containing derivative chromosome 9 and derivative chromosome 11 and physically localized both breakpoints to small intervals. We used these resources and available partial genomic sequences in this region of chromosome 11q23.1 to localize the breakpoint precisely. We found that the breakpoint at 11q23.1 occurred in a repeat-rich region. A number of Alu and L1 type repeat elements immediately flanked the breakpoint at either side. The breakpoint mapped at the immediate 5'-end of a partial Alu sequence. Using the sequence information from the chromosome 11q23 breakpoint region, we captured chromosome 9 sequences flanking the breakpoint. First, we prepared bubble-vectorette libraries from hybrid cell lines and then performed PCR amplifications using primers designed from the known chromosome 11 sequences and the vectorette sequence. We found that the breakpoint at chromosome 9p24 occurred within an L1 type repeat element. Because the genomic regions at 11q23 and 9p24 involved in the translocation have no apparent similarity, the translocation is likely to be a result of non-homologous recombination. Comparison of the sequences of the derivative chromosomes with those of the normal BAC clones indicates that no genomic loss occurred during the translocation. These results suggest that Alu or L1 repeats might be responsible for this constitutional chromosomal rearrangement and provide us with the exact positional information to search for gene(s) on either chromosome that might be disrupted by the translocation breakpoints.

Identification of functionally homologous genes in two regions on chromosome 15 associated with cytogenetic abnormalities in autism. *M. Smith, C. Wu, J. Escamilla, P.A. Filipek, M.E. Bocian, M.A. Spence.* Dept Pediatrics, Univ California, Irvine, Irvine, CA.

We have described a patient with autism and a microdeletion of the 15q22-q23 region .(Smith et al, In press Amer. J. Med. Genet). This patient had several clinical features suggestive of Angelman syndrome. The patient had white-blond hair, light blue lacy irises and skin, hair and eye color was much lighter than that of her parents. The craniofacial examination revealed somewhat flattened occiput, thin upper lip, prominent jaw and pointed chin. There was a history of delayed developmental milestones. It is likely that the clinical features and autistic behaviors in our patient are due to deletion of a specific gene or genes. Of interest is whether or not genes which map within the deletion region defined in our patient are homologous to genes in other chromosomal regions which are deleted, duplicated or rearranged in autism. There is evidence from linkage studies and from cytogenetic studies that a locus on chromosome 15q11-15q13 plays a role in development of autism, Schroer et al. (1998). Steffenberg (1996) described four patients with childhood autism and Angelman syndrome. Our recent analysis of the map positions of genes in the 15q21-q23 region and our analysis of sequenced bac contigs reveals that a number of genes within the 15q11-13 and within 15q21-q23 region show significant functional homology. The Herc 2 gene and its duplcons maps to 15q11 q13, Herc1 maps to 15q22 (Rosa et al 1999). A Hect domain (characteristic of Herc genes) is also present in the NEDD4 gene (neural precursor cell expressed), Kumar et al 1997). We recently determined that this gene is contained within Bac AC09997 which maps to the chromosome 15q21.3 region. The Angelman gene ubiquitin ligase E3A (UBE3a) maps to 15q12. Ubiquitin ligase E3 alpha which functions in the N-end rule pathway of ubiquination (Kwon et al. 1998) maps to 15q21. Functionally homologous genes which map to the two regions of chromosome 15 which show cytogenetic abnormalities in autism are candidate genes for autism.

Neuronal intranuclear accumulation of mutant DRPLA protein with expanded polyglutamine stretches and transcriptional dysregulation in DRPLA transgenic mice (Q129). *T. Sato*¹, *M. Yamada*², *M. Oyake*¹, *T. Shimohata*¹, *K. Nakao*³, *K. Nakamura*³, *M. Katsuki*³, *H. Takahashi*², *S. Tsuji*¹. 1) Dept. of Neurology; 2) Pathology, Brain Research Inst., Niigata Univ., Niigata; 3) Center for Experimental Medicine, Inst. of Medical Science, Univ. of Tokyo, Japan.

To create animal models for dentatorubral-pallidoluysian atrophy (DRPLA), we generated transgenic mice harboring a single copy of a full-length human mutant DRPLA gene with 129 CAG repeats (AJHG 65(suppl): A30, 1999). Although Q129 mice exhibited a severe phenotype, progressive reduction of the entire brain and intranuclear accumulation of mutant DRPLA protein, neuronal loss was not observed, suggesting that neuronal dysfunction is responsible for the disease expression. Hypothesizing that neuronal intranuclear accumulation leads to neuronal dysfunction, we performed expression profiling of Q129 mice. We analyzed expression profiles (~11000 genes) in brains of Q129 and non-transgenic mice at 4, 8 and 12 weeks of age using oligonucleotides micro-arrays (Affimetrix).

We identified approximately ~40 affected genes. Many of them were down-regulated in Q129 mice and became more prominent with age. The down-regulated genes included those involved in neuronal plasticity, such as cAMP- and retinoid-responsive genes (enkephalin, *Egr1*, *N10*, somatostatin), or in calcium homeostasis (*SERCA2*). Down-regulation of a distinct gene set suggests neuronal dysfunction caused by expanded polyQ stretches. Furthermore, there were many similarities in the affected genes compared with those reported for SCA1 and R6/2 HD mice. These results suggest that common mechanisms are involved in the pathogenesis of polyglutamine diseases. We have recently found that expanded polyQ stretches strongly bind to a TATA-binding protein-associated factor, TAFII130, which is a coactivator of cAMP responsive element-binding protein (CREB1). Since many of the down-regulated genes contain cAMP-responsive elements, present study raised an intriguing possibility that binding of expanded polyQ stretches to TAFII130 is involved in the neuronal dysfunction in polyglutamine diseases.

Trinucleotide Repeat Expansions implicated in SCA in a large group of families and isolated cases with ataxia. I.

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The spinocerebellar ataxias (SCAs) are neurodegenerative disorders clinically and genetically very heterogeneous. Nine genetically different SCAs are already known caused by trinucleotide repeat expansions (TRE). For the dominant SCAs the mutant proteins show an expanded polyglutamine tract in SCA1, SCA2, MJD/SCA3, SCA6, SCA7, and DRPLA, while SCA8 and SCA12 are caused by untranslated (CTG)_n and (CAG)_n expansions, respectively. In Friedreich ataxia, a recessive SCA, the mutant protein is deficient due to a (GAA)_n expansion in intron 1 of the FRDA gene. In order to identify the genetic defect, we have looked for these TREs in our families. We studied 201 Portuguese and Brazilian unrelated individuals with SCA, belonging to 102 families with dominant inheritance, 23 with apparently recessive transmission and 76 isolated cases. Of families with dominant inheritance, 74% had (CAG)_n expansions at the SCA2 (3%), MJD (65%), SCA6 (1%), SCA7 (1%) or DRPLA (2%) loci, and 2% showed (CTG)_n expansions at the SCA8 gene; 70% of the recessively transmitted cases had a (GAA)_n expansion. Among isolated cases, 11% showed (CAG)_n expansions at the MJD locus, 4% showed (CTG)_n expansions at the SCA8 gene and 15% had (GAA)_n expansions. No (CAG)_n expansions at the SCA1 or SCA12 loci were found. In conclusion, SCAs in our population of patients are mostly due to (CAG)_n, followed by (GAA)_n expansions; however, in almost half of our patients with SCA the molecular basis remains unknown.

Genetic analysis of the Milwaukee Longitudinal Cohort Study of Attention Deficit Hyperactivity Disorder. *K.M. Smith¹, Y. Kleyner¹, M.J. Daly², E. Lander², R. Barkley³, B.A. Navia^{1,4}*. 1) Gen Prog/Sackler Sch GBS, Tufts Univ, Boston, MA; 2) Whitehead Institute, MIT, Cambridge, MA; 3) Dept. of Psychiatry, UMass Medical Center, Worcester MA; 4) Neurology Dept., Research Laboratories in Psychiatry, NEMC, Boston, MA.

Attention Deficit Hyperactivity Disorder (ADHD) is a highly inheritable and common disorder. Polymorphisms in or near genes involved in dopamine signaling and metabolism have been associated with ADHD, including the 7 repeat allele of the D4 Dopamine Receptor (D4DR) exon 3 VNTR, and the 480 bp allele of the 3'UTR VNTR of the Dopamine Transporter Gene (DAT1). We have initiated genetic studies of candidate genes in a unique cohort of 158 ADHD and 81 age-matched controls who have been followed as participants in the Milwaukee longitudinal study of ADHD. Affected individuals were diagnosed during childhood and have been evaluated at 5 year intervals for the past 20 years with measurements of ADHD symptom levels and various assessments of psychological, behavioral, educational, social and daily adaptive functioning. The combined genotype-phenotype studies in this cohort will allow greater understanding of the effects of these candidate alleles on the different developmental outcomes of ADHD.

A pilot genetic study of 66 Caucasian participants with ADHD and 30 age and ethnicity matched controls has been initiated. These individuals were genotyped for the D4DR exon 3 VNTR, the DAT1 3'UTR VNTR and the Dopamine β Hydroxylase (DbH) GT repeat polymorphisms. Preliminary results indicate a trend towards an increased frequency of the DbH A4 allele in the ADHD group ($.10 > p > .05$), suggesting that this allele, or another polymorphism in linkage disequilibrium, may confer increased susceptibility towards ADHD. This allele has been shown to be associated with high enzyme activity, consistent with the hypothesis that a hypodopaminergic state is present in affected individuals. Further studies are underway to determine the association of these polymorphisms singly and in combination, to various phenotypic outcomes of ADHD.

Loss of methylation at KvDMR1 is a frequent epigenetic change in Beckwith-Wiedemann syndrome. *C.D. Day¹, N. Diaz-Meyer¹, C. Junien², V.M. Der Kaloustian³, G.E. Graham³, P.J. Bridge³, E.M. Algar⁴, E.R. Maher⁵, T.B. Shows¹, M.J. Higgins¹.* 1) Roswell Park Cancer Inst, Buffalo, NY; 2) Inserm UR383, Hopital Necker Enfants Malades, Paris, France; 3) Montreal and Alberta Children's Hospitals; 4) University of Melbourne, Melbourne, Australia; 5) University of Birmingham, Birmingham, UK.

Beckwith-Wiedemann syndrome (BWS) is a phenotypically variable disorder characterized by generalized overgrowth, congenital abnormalities, and a predisposition to embryonal cancers. BWS can result from multiple genetic and epigenetic mechanisms, most appearing to deregulate normal imprinted expression on chromosome 11p15. BWS is also referred to as EMG syndrome, an acronym for its major phenotypic characteristics of exomphalos, macroglossia, and gigantism. However, several other overgrowth syndromes have phenotypes that overlap with BWS making differential diagnosis difficult in some cases. We recently described a novel epigenetic abnormality in 5 of 12 patients with BWS consisting of the loss of imprinted methylation at a differentially methylated CpG island (KvDMR1) that maps to 11p15 (Smilinich et al. PNAS 96: 8064, 1999). This finding raised the possibility that assessment of KvDMR1 methylation might facilitate the differential diagnosis of overgrowth syndrome patients. We have now analyzed KvDMR1 methylation status in 115 patients with BWS and biparental inheritance of 11p15 by Southern hybridization analysis of KvDMR1 using the methylation-sensitive restriction enzyme NotI. DNA from 35 patients exhibited complete loss of methylation at KvDMR1, while an additional 24 showed "partial" loss of methylation at this locus. Twenty-four (24) of these patients were also analyzed for methylation at the H19 promoter with four exhibiting hypermethylation at H19; none of these four patients showed loss of methylation at KvDMR1 consistent with our earlier hypothesis that loss of methylation at KvDMR1 is an independent epimutation. In summary, either complete or partial loss of methylation at KvDMR1 occurs in more than 50% of non-UPD (uniparental disomy) patients with BWS and may serve as a useful diagnostic marker for this condition. NIH CA63333.

Functional analysis of KvDMR1, a putative imprinting control region in mouse distal chromosome 7. G.V.

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A frequent (about 50 % non-UPD patients) epigenetic abnormality in individuals with BWS is evidenced by the loss of imprinting at a differentially methylated CpG island (KvDMR1) which maps within a large intron of the paternally imprinted *KCNQ1* gene in human 11p15.5 and mouse distal chromosome 7 (Smilinich et al. PNAS 96: 8064, 1999). KvDMR1 also contains a direct repeat structure and is associated with a large paternally expressed untranslated RNA transcript and is thus reminiscent of several loci believed to be cis-acting imprinting control regions (ICRs). Some ICRs have been proposed to function as chromosome insulators that, in an allele-specific manner, can block the communication between a gene promoter and an enhancer. KvDMR1 was tested for this activity by inserting it between the promoter of a reporter gene and an SV40 enhancer in an episomal construct and carrying out a transient expression assay. A ribonuclease protection assay demonstrated that the KvDMR1 sequence repressed SV40 enhancer-directed expression of the reporter gene 5-10-fold in a position-independent manner, suggesting that, in this context, this locus can act as a chromosome insulator and/or silencer. To distinguish between these possibilities KvDMR1 is being tested further as an insulator element in a colony assay where constructs are integrated into the genome. To determine which gene or genes KvDMR1 might be controlling, the locus was deleted by homologous recombination in ES cells and strains of mice generated carrying the deleted allele. The mutant allele has been transmitted both maternally and paternally into a polymorphic background and molecular analyses of distal chromosome 7 imprinted genes are underway to assess any disruption of genomic imprinting. Funded by NIH/NCI Grant CA63333.

Decreased apoptosis in Epstein-Barr virus (EBV)-immortalized lymphocytes from normotensive young males possessing the G-protein beta 3 subunit C825T variant. *Y.B. Dong^{1,2}, S. Rizzo³, J.F. Murray², A. Ion², H.D. Zhu¹, F.M. Gibson³, N.D. Carter².* 1) Blood Pressure Unit; 2) Medical Genetics Unit; 3) Department of Haematology, St. George's, Hosp Med School, London, UK.

Previously, we have shown that the C825T polymorphism of the beta 3 subunit of the PTX-sensitive Gi type proteins has a population impact on the risk of hypertension and the T allele was also found to contribute to hypertension-related disorders such as obesity and left ventricular hypertrophy. The aim of the present study was to investigate the effect of the T allele on the pattern of cell apoptosis and growth in EBV-immortalised lymphocytes. Methods: 18 healthy, young individuals, including 6 CC, 6 CT, 6TT, were selected for this study. Lymphocytes from the individuals were immortalized with EBV. All cell lines were re-seeded at 1×10^4 cells /ml on day 0 and grown for 2 days. Cell numbers were counted on each day. On day 1 and day 2, 1×10^4 cells from each cell line were incubated with 50 ml of 7AAD on ice for 20 min in the dark and analysed by flow cytometry. Negative (live), dim (apoptotic), bright (dead) cell populations were respectively quantified by 7AAD fluorescence using Cell Quest software. Results: The mean ratio of cell counts on day1/day 0 and day 2/day 0 significantly increased in individuals with TT compared to CC (day1/0: CC 1.33 vs.TT 1.61, $p < 0.0001$; day2/0: CC 2.32 vs.TT 2.68, $p < 0.0001$). 7AAD analysis revealed that there were significantly fewer apoptotic cells in the TT group when compared in the CC group on both days ($p < 0.0001$). Apoptotic cell numbers decreased with increasing numbers of T alleles by day 1: CC 1.46%, CT 1.19%, TT 0.52%; and day 2: CC 1.77%, CT 1.15%, TT 0.87%. Conclusions: Possession of the G protein 825T allele is associated with increased rate of cell growth and decreased apoptosis in EBV-immortalized lymphocytes, which is secondary to upregulation of G protein activity. A large proportion of human populations studied possess the T allele, which may contribute to common diseases such as hypertension and obesity.

Genetic dissection of Familial Combined Hyperlipidemia. *P.M.H. Eurlings, J.M.W. Geurts, R.G.J.H. Janssen, F.G. Bouwman, T.W.A. de Bruin.* Internal Medicine, Maastricht University, Maastricht, Limburg, The Netherlands.

Familial Combined Hyperlipidemia (FCHL) is the most common inherited hyperlipidemia in the Western Caucasian population, with a frequency of 1~2% in the general population and approximately 10% in survivors of premature myocardial infarction. In order to dissect the genetic background of this complex trait we report two lines of research. Differential gene expression is studied in adipose tissue samples of FCHL patients and matched controls, using Atlas cDNA expression arrays (Clontech; PT3140-1). Initial analyses resulted in the identification of 22 differentially expressed genes, representing predominantly receptors, cell-surface antigens, and cell adhesion molecules. It should be noted that the observed FCHL expression profiles require further validation. Additionally, we generated differential cDNA libraries, using suppression subtractive hybridization (SSH), providing another powerful research tool. Expression and conceptual FCHL candidate genes are examined in more detail in association studies and screened for mutations. Data obtained for the PPARA gene through this approach showed an overall significant difference in allelic distribution between FCHL patients and controls ($p=0.006$), using CA-repeat marker D22S1144. This difference was due to an enrichment of allele CA20 in the FCHL group. Moreover, we identified several mutations in the PPARA coding sequences, i.e. a L162V mutation in the DNA binding domain. These data combined indicate the involvement of PPARA in the development of FCHL.

b-2 adrenergic receptor gene variations and blood pressure under stress in normal twins. A. Busjahn¹, G.-H. Li², H.-D. Faulhaber¹, M. Rosenthal¹, H. Schuster¹, J. Jordan¹, B. Timmermann², M.R. Hoehe², H. Knoblauch¹, F.C. Luft¹. 1) Dept Nephrology/Hypertension, Franz Volhard Clinic, Berlin, Germany; 2) Max-Delbrück-Center for Molecular Medicine, Berlin, Germany.

Complex traits like hypertension do not follow simple mendelian inheritance. In these cases, studying genetic influences on regulatory pathways may be more fruitful than looking at clinical endpoints. For blood pressure as a very dynamic trait heritability for situational adaptation has been demonstrated by twin studies. We tested the hypothesis that blood pressure responses to physical and mental stress are associated with polymorphisms in the b-2 adrenergic receptor gene. We studied normotensive, young, monozygotic (MZ) and dizygotic (DZ) twins. The subjects underwent automated blood pressure measurements at the brachial and digital arteries and were subjected to mental arithmetic and cold pressor stress. We used allele-specific PCR to genotype the subjects in terms of four single nucleotide polymorphisms in the b-2 AR gene. The most functionally-relevant polymorphism in the b-2 AR gene, namely Arg16/Gly, was associated with systolic and diastolic blood pressure under resting conditions, during mental arithmetic, and during the cold pressor test, as well as with the increase in diastolic blood pressure during both forms of stress. These findings support a role for the b-2 AR gene in blood pressure regulation. They also indicate that the b-2 AR gene influences the level of not only resting but also stress-related blood pressure.

Multidrug Resistance (MDR) Genetic Locus Influences Circulating Triglyceride Levels in a Population with Coronary Heart Disease. *D.W. Neklason, B.E. Otterud, H.H. Coon, S.C. Hunt, J.E. Metherall.* Departments of Human Genetics, Psychiatry and Cardiovascular Genetics, Internal Medicine, University of Utah, Salt Lake City, UT.

The activity of the multidrug resistance (MDR) P-glycoproteins is required for 1) cellular cholesterol biosynthesis, 2) cholesterol esterification, and 3) the secretion of bile. These biochemical and physiologic observations led us to ask if inherited abnormalities in MDR activity contribute to the development of coronary heart disease (CHD) by ascertaining coinheritance at the MDR locus in sibling pairs affected with CHD. Five polymorphic genetic markers linked to the MDR locus on chromosome 7q21 and spanning 12.3 cM were evaluated in 175 individuals composing 106 sibships from 81 independent families. No linkage disequilibrium was evident, and there was no increase in shared alleles in sibling pairs with CHD. These results suggest that inherited abnormalities at the MDR locus do not cause CHD. We next investigated whether the MDR locus influenced circulating lipid profiles in the sibling pairs. We tested age of onset, high cholesterol, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, and VLDL cholesterol levels, and age- and sex-adjusted categories of total cholesterol, triglycerides, HDL, and LDL. Multipoint Haseman Elston analysis revealed a correlation between inheritance at this locus and age-and sex-adjusted circulating triglyceride levels ($p = 0.009$) and high cholesterol levels ($p = 0.028$) in the CHD sibling pairs. A model is presented where inherited variations in MDR activity affect absorption of dietary cholesterol and triglyceride rich fats.

Polymorphisms in the angiotensinogen gene are associated with intima-medial wall thickening in females from a community based population. *C.M. Chapman¹, B.M. McQuillan^{1,4}, J. Hung^{1,4}, J.P. Beilby¹, L.J. Palmer^{2,3}.* 1) Clinical Biochemistry, PathCentre, QEII Med Center, Perth, Australia; 2) Channing Laboratory, Brigham & Women's Hospital and Harvard Medical School, Boston, MA; 3) Dept Epidemiology & Biostatistics, Case Western Reserve University, Cleveland, OH; 4) Heart Research Institute of Western Australia, Perth, Australia.

Polymorphisms in genes within the renin angiotensin system (RAS) have been associated with an increased risk of cardiovascular disease. We investigated the association of polymorphisms in the angiotensinogen and angiotensin II receptor type I genes with increased carotid intima-medial wall thickness (IMT) and the presence of plaques in carotid arteries in a randomly selected sample of Australian adults. Three polymorphisms were genotyped: two in the promoter of the angiotensinogen gene, Ang G-6A, Ang A-20C; and one in the angiotensin II receptor type 1 gene, ATRI A1166C. Using multivariate generalised linear models, the Ang -6 A allele ($p < 0.001$) and the Ang 20 C allele ($p < 0.03$) were found to be significantly associated with increased mean carotid IMT in females but not in males when adjusted for conventional risk factors. The ATRI A1166C polymorphism did not show any significant relationship to mean IMT. There was some evidence that the I allele of the angiotensin converting enzyme (ACE) insertion/deletion (ID) polymorphism interacted epistatically with the Ang G allele to increase mean carotid IMT in the population as a whole. None of the polymorphisms investigated were significantly associated with the presence of carotid plaques. This study has shown for the first time strong sex- and age-dependent effects of RAS genetic polymorphism on an important cardiovascular phenotype.

Sequence variants in a 14.4-kb region of the human angiotensinogen gene for more details of molecular etiology of essential hypertension. *T. Nakajima*¹, *T. Ishigami*², *I. Nakazawa*², *M. Emi*³, *S. Umemura*², *I. Inoue*¹, *J-M. Lalouel*⁴.

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Sequence variation in human genes is largely confined to single-nucleotide polymorphisms (SNPs) and is valuable in test of association with common diseases. Linkage and association studies have shown that molecular variants in the human angiotensinogen gene (AGT) contribute inherited predisposition to essential hypertension. In previous studies we reported that the T235 polymorphism encoding a threonine instead of a methionine at residue 235 of the mature angiotensinogen protein and a molecular variant in the proximal promoter of AGT, an adenine instead of a guanine six nucleotide upstream from the site of transcription initiation site (A(-6)) were significantly more common in hypertensive subjects than in normotensive controls and associated with a 10%-20% increase in plasma angiotensinogen. T235 and A(-6) were in very tight linkage disequilibrium. In this study we determined the complete genomic sequence of human AGT and performed the contiguous scan (14.4 kb) for sequence variation in human DNA. In total, forty single nucleotide polymorphisms and a dinucleotide repeat sequence were identified from 31 human chromosomes. Among single nucleotide polymorphisms, transition substitution were more prevalent (35 of 40, 87.5%) than transversions (15 of 40, 37.5%). The majority of variable sites were found in the non-coding regions (38 in the non-coding, 2 in the coding). The insertion/deletion variants except for a dinucleotide repeat were not present in this region. Eight of them are in tight linkage disequilibrium with T235 and A(-6). Those variants could be causally implicated, alone or in combination with A(-6) in the development of essential hypertension and the determination of plasma angiotensinogen level.

The genetics of stroke. A.C. Morrison¹, A.R. Folsom³, M. Fornage², E. Boerwinkle^{1,2}. 1) University of Texas-Houston, Houston, TX; 2) Institute of Molecular Medicine, Houston, TX; 3) University of Minnesota, Minneapolis, MN.

Stroke is the leading cause of disability and the third leading cause of death in the United States. Nevertheless, few studies have attempted to identify and characterize the role of particular genes in the etiology of stroke in the general population. To this end, we have investigated the familial aggregation of stroke to characterize the role of shared genetic factors, conducted association studies of *a priori* biologic candidate genes, and performed genome-wide linkage analyses to identify chromosomal regions that show linkage with disease-related phenotypes.

Unrelated individuals with subclinical cerebral infarction and incident clinical ischemic stroke were identified in the Atherosclerosis Risk in Communities (ARIC) Study. Parental history of stroke was significantly associated with subclinical stroke after adjusting for age, gender and race ($p < 0.01$), and for stroke risk factors ($p < 0.01$). Genome-wide linkage analyses in samples of hypertensive sibling pairs are used to identify novel genes contributing to a positive family history of stroke. Studies of biologic candidate genes are being pursued to support this genome-wide effort. The G-protein β_3 subunit (GN β_3) C825T and lipoprotein lipase (LPL) Ser447Ter polymorphisms were genotyped in the ARIC Study subclinical and clinical cases. The GN β_3 825T allele was significantly associated with clinical stroke in Whites after adjusting for age and gender ($p < 0.05$), and for stroke risk factors ($p < 0.05$). The LPL 447Ter allele was significantly associated with subclinical stroke ($p < 0.05$) and marginally associated with clinical stroke ($p = 0.06$) in men after adjusting for age and race. After adjusting for stroke risk factors, the LPL 447Ter allele significantly predicted both subclinical and clinical stroke in men ($p < 0.01$).

Identification of genes contributing to stroke risk may be useful for the early identification of individuals at increased risk for stroke prior to the onset of clinical symptoms and for developing a better understanding of the etiology and pathophysiology of the disease.

Genetic controls of homocysteine metabolism in mice: interacting pathways and expression profiles. J.H.

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Elevated homocysteine is a risk factor for cardiovascular disease and neural tube defects. Several genetic factors, including an MTHFR polymorphism, account for some of these risks. However, polymorphisms in MTHFR, CBS, MS and MTRR do not account for the various diseases and birth defects associated with hyperhomocysteinemia. To identify mouse models for studying the causes and consequences of hyperhomocysteinemia, we surveyed homocysteine levels in a panel of mutant mice with birth defects or adult diseases similar to those associated with hyperhomocysteinemia in humans. Many single gene mutations showed differences in homocysteine levels in mutants versus their wild-type controls. Many of these mutations involve genes in the hedgehog and WNT / disheveled signal transduction pathways. Expression profiles were characterized with liver samples from these mutant and control mice together with arrays that include most of the genes in the folate and homocysteine pathways. Most of the genes showing significant differences in expression patterns in these mutant versus control comparisons were involved in the folate and homocysteine pathways. Together these results suggest that mutations in the hedgehog and WNT / Disheveled pathways adversely affect folate and homocysteine metabolism.

The angiotensinogen 235T common polymorphism increases the risk of myocardial infarction in patients with coronary artery disease. *C. Stranieri¹, O. Olivieri², D. Girelli², E. Trabetti¹, R. Corrocher², P.F. Pignatti¹.* 1) Sect Biol & Genetics, Dpt. Mother-Child, Biol & Genetics, Univ. Verona, Italy; 2) Dpt. Clin. Exp. Med., Univ. Verona, Italy.

The angiotensinogen (AGT) and the Angiotensin II type 1 receptor (ATIIR) genes have been associated with the risk of coronary artery disease (CAD). The AGT M235T polymorphism correlates with an increased plasma AGT concentration, and the ATIIR A1166C polymorphism increases the response to ATII. A total of 699 subjects, were genotyped for the 2 polymorphisms: 454 with and 245 without angiographically documented severe CAD. Among the patients with coronary disease, 247 had experienced myocardial infarction (MI). AGT M235T and ATIIR A1166C allele and genotype frequencies were similar in CAD and CAD-free patients. In the CAD group, the AGT 235T allele resulted more frequent in subjects who had experienced myocardial infarction (0.49 vs 0.41; $P < 0.05$). No allele frequency difference was observed for the ATIIR polymorphism in CAD subjects with or without MI (allele 1166C is 0.28 in both classes). By logistic regression, homozygosity for the AGT 235T variant appeared to confer a 1.9-fold increased risk for MI in subjects with documented severe coronary artery disease.

A common IL-6 gene promoter polymorphism frequency analysis in unstable angina patients. *P.F. Pignatti¹, C. Stranieri¹, G. Liuzzo², D.J. Angiolillo², L.M. Biasucci²*. 1) Sect Biol & Genetics, Dpt.Mother & Child, Biol & Gen, Univ., Verona, Italy; 2) Cardiology, Catholic University, Rome, Italy.

The evidence of elevated levels of acute-phase proteins in unstable angina suggests that inflammation plays a pivotal role in acute coronary syndromes and is an indirect sign of the increased production of interleukins. Interleukin-6 (IL-6), acting on hepatocytes, may induce acute-phase reactants that increase blood viscosity and promote thrombus formation. In the 5' region of the human IL-6 gene the common polymorphism G-174C has been described. In healthy subjects, the G allele was associated with higher levels of plasma IL-6. We have collected 58 patients with severe unstable angina (i.e. resting angina in the last 48 hrs, Braunwald class IIIB) and 17 patients with stable angina. All the study subjects (n=75) were genotyped for the G-174C polymorphism. In unstable angina, 36/58 (62%) G homozygotes were detected, compared to 7/17 (41%) in stable angina. After LPS stimulation of monocytes in vitro, G homozygote patients had higher IL-6 levels (n=15, median 4435 pg/ml) than heterozygotes (n=8, median 2000 pg/ml), $P=0.05$. The frequency of the G allele was higher in unstable angina compared to stable angina patients (81% v 62%, respectively, $P=0.02$). These data indicate that the IL-6 gene may be involved in the pathogenesis of unstable angina.

Interaction of the Paraoxonase Genes PON1 and PON2 and the methylenetetrahydrofolate reductase (MTHFR) gene and association with angiographic coronary artery disease. *M.P. Reilly¹, M.L. Wolfe¹, L. Picoult-Newberg², A.S. de Lemos¹, E.R. Mohler¹, M. Pohl², W. Matthai¹, R. Wilenski¹, D.M. Grant², M. Boyce-Jacino², D.J. Rader¹.* 1) University of Pennsylvania, Philadelphia, PA; 2) Orchid BioSciences, Inc. Princeton, NJ.

Serum paraoxonase (PON) is an HDL-associated enzyme that inhibits LDL oxidation. PON has recently been shown to exhibit homocysteine thiolactonase activity, implicating it in the breakdown of pro-atherogenic homocysteine metabolites. PON may therefore provide protection against the risk of coronary artery disease (CAD). Three PON genes, designated PON1, PON2 and PON3, have been identified, and polymorphisms in the PON1 (Gln192Arg) and PON2 (Ser311Cys) genes have been associated with risk for CAD. We undertook a study of 493 Caucasians (103 controls: <10% stenosis; 390 cases: >50% stenosis) in which we examined the association of these two PON polymorphisms with angiographic CAD and their interaction with a polymorphism in the MTHFR gene (Ala226Val) that is associated with elevated plasma levels of homocysteine. We used a single-base primer extension method (SNP-IT™ Single Nucleotide Polymorphism Identification Technology) for high-throughput genotyping of DNA samples. We found no significant difference in the allele frequencies of PON1 Arg192 (31.3% vs 26.2%; $p=0.19$) and PON2 Ser311 (79.0% vs 79.1%; $p=0.96$) between cases and controls. Stratification of the PON1 data by the presence or absence of the PON2 Ser311 allele, however, showed a significant difference in the PON1 Arg192 allele frequency between cases and controls (32.2% vs 22.1%; $p<0.05$). The MTHFR Val226 allele frequency was less common in cases than controls (35.2% vs 47.4%; $p<0.05$). Furthermore, the frequency of MTHFR Val226 in cases and controls was not affected by stratification of MTHFR data by the presence of the PON1 Arg192 allele (35.9% vs 47.3%) or the PON2 Ser311 allele (35.7% vs 47.3%). Our results suggest that single nucleotide polymorphisms in PON1 and PON2 additively contribute to the risk of CAD, whereas neither of the PON gene variations has an influence on the relationship of MTHFR Ala226Val gene polymorphism to the risk of CAD.

Xenomitochondrial mouse cell models of mtDNA disease: Impaired respiratory chain function, increased H₂O₂ output, and transfer to mouse ES cells. I.A. Trounce, M. McKenzie. Mutation Research Ctr and University of Melbourne, Melbourne, Australia.

The production of mouse models of mtDNA defects will benefit studies of pathogenesis and inheritance of mtDNA diseases, and provide new tools for the study of complex Mendelian disorders with mitochondrial effects. Methods to produce targeted mtDNA mutations have not yet been reported, and few mouse cell mtDNA mutants are described. We have produced three constructs by placing mtDNAs from *Mus spretus*, *Mus dunni* and *Rattus norvegicus* into *Mus musculus* r⁰ cells, producing respiratory chain defects ranging from severe complex I and III defects in the *Rattus* cybrids (Trounce & McKenzie, AJHG 65:A431, 1999), to a mild complex III defect in the *M. dunni* cybrids. A microplate fluorimetric assay measuring H₂O₂ production has been developed to directly follow *in vitro* reactive oxygen species (ROS) output associated with the enzyme defects. We show that H₂O₂ output is increased by 120% in the *Rattus* cybrids at both respiratory chain complex I and III, producing the first evidence of increased ROS output consequent to an mtDNA respiratory chain defect. In the *M. dunni* cybrids this output is increased by 50% at complex III only, while overall respiratory chain electron flux is not significantly reduced. A subtle respiratory chain defect was indicated in the *M. dunni* cybrids by a modestly increased lactate production. To test the potential of this xenocybrid system to produce mice, transfer of the foreign mtDNAs into *M. musculus* embryonic stem (ES) cells was undertaken by pre-treatment of the ES cells with rhodamine 6G. Both *M. spretus* and *M. dunni* mtDNAs were successfully transferred into the ES cells, showing that homoplasmic transfer is possible without the use of selectable mtDNA markers such as chloramphenicol resistance. We have been unable to obtain non-differentiated xenocybrid ES cells with *Rattus* mtDNAs, suggesting that the defects in this construct are too severe to allow normal ES cell growth. In the absence of more directed mtDNA mutants, the production of xenomitochondrial mice should allow the first dissection of pathologic effects resulting from energetic and ROS defects.

A Further Case of the Angelman Syndrome Molecular Defect Presenting with an Atypical Phenotype. *N. Clarke¹, T. Roscioli¹, P.J. Taylor², M-Y. Yip², J. Jackson³, M.F. Buckley², A. Turner¹.* 1) South Eastern Sydney Genetics Service, The New Children's Hospital, Sydney, NSW, Australia; 2) Molecular and Cytogenetics Unit, South Eastern Area Laboratory Services, Randwick, NSW 2031; 3) Molecular Genetics Laboratory, Western Sydney Genetics Program, New Children's Hospital, Westmead, NSW, 2145.

We present a case of the absence of a maternally imprinted copy of the SNRPN locus on chromosome 15q consistent with Angelman syndrome (AS) in a boy initially diagnosed with Borjeson-Forssman-Lehmann syndrome (BFLS). BFLS is a rare X-linked condition that is characterised by moderate to severe developmental delay, tapered fingers, prominent ear lobes and small genitalia. Other features usually seen in BFLS include epilepsy and wide gaps between the toes. This 11 year old boy presented with moderate obesity (weight, 97th centile) and head circumference and height between the 25th and 50th centiles. He had mildly coarse facial features, a broad nasal tip, long ears with thickened lobes, tapered fingers, curved toes, small genitalia and gynecomastia. Although he had only 4 words, he understood simple sentences. His epilepsy was well controlled and he lacked the typical hand movements, ataxia and spontaneous laughter that are associated with AS. A karyotype and Fragile XA analysis were normal and HbH bodies were not found. During investigations for Prader-Willi syndrome molecular analysis detected the absence of a maternally imprinted allele at the SNRPN locus, a finding which is characteristic of AS. FISH analysis using a probe for SNRPN for the Prader-Willi and AS region on 15q demonstrated the lack of a deletion. These unexpected findings are consistent with the findings of Gillessen-Kaesbach et al., (1999) of a subset of AS patients with normal stature and head circumference, obesity, absence of typical facial features and less severe developmental delay than is usually seen in AS. Additional elucidation of the molecular aetiology of this boys condition is to follow. This result may be of assistance in determining the aetiology of children with a similar phenotype.

Program Nr: 1923 from the 2000 ASHG Annual Meeting

Multiplex Thrombophilia Testing Using the Li-Cor™ Automated DNA Sequencer. *D. Kennedy, C.R. Pereira, A. Rezaie, M. Skuterud, J. Steer, J.P. Tzountzouris, D.J. Allingham-Hawkins.* Molecular Genetics, North York General Hosp, North York, ON., Canada.

Factor V Leiden, prothrombin G20210A, methylenetetrahydrofolate reductase (MTHFR) C677T and cystathionine beta-synthase (CBS) 844ins68 are genetic variants found in all human populations. Factor V Leiden and prothrombin G20210A confer a moderate increase in risk of deep vein thrombosis and may also contribute to the risk of obstetrical complications and superficial thrombophlebitis. MTHFR C677T may confer a moderate increase in risk of premature coronary artery disease as well as contribute to the risk of obstetrical complications such as pre-eclampsia. CBS 844ins68 may also increase the risk of premature coronary artery disease especially in combination with MTHFR C677T. We have developed a multiplex assay for these four mutations with detection using the Li-Cor™ automated DNA sequencer. This assay allows rapid analysis of all four variants in a single lane, increasing throughput and decreasing turnaround time. A risk can then be given taking all of the information into account. To date, 13 variants have been detected among 14 samples. The incidence of the variants detected to date is 11% for Factor V Leiden, 0% for prothrombin G20210A, 25% for MTHFR C677T and 11% for CBS 844ins68. The method will be presented along with updated data from our testing program.

Characterization of dFXR: the fly homologue of FMR1. *J. Morales¹, B. Hassan^{1,2}, H. Bellen^{1,2}, D.L. Nelson¹.* 1) Dept Molecular & Human Gen, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute, Baylor College of Medicine.

The molecular basis of Fragile X Syndrome remains elusive despite nearly ten years of analysis of the FMR1 gene. A number of animal models, including an FMR1 KO mouse and several transgenic mouse lines, have not provided significant insight into the pathogenesis of the disease. Therefore, the study of this gene product in a less complex system has been of great interest. The completion of the Drosophila Genome Project has facilitated this aim by uncovering the fly homologue of FMR1 (dFXR) and by generating a number of mutant lines. The Drosophila protein is highly conserved with an overall amino acid identity of 35% with the mammalian version. Identity in the RNA binding domains (60%) also suggests functional conservation. The aim of this study is the characterization of dFXR and its genetic pathway. In Situ Hybridization experiments indicate that the pattern of expression is ubiquitous during early stages of development, similar to humans and mice. Northern analysis and RT-PCR are being used to study the adult. Human and mouse antibodies fail to cross react with the fly homologue, therefore, GFP fusion proteins and antibodies are being generated to study protein expression and intracellular location. dFXR mutant flies were obtained from the laboratory of G. Rubin and genetic tools have been used to characterize their phenotype. Surprisingly, male specific lethality was associated with some P-element excision lines and efforts are underway to understand the cause of this remarkable phenotype. Additionally, wild type and I307N dFXR were over expressed in the wing, thorax and a collection of brain cells. Generalized abnormalities were present when both proteins were over expressed but a milder phenotype was observed for the point mutation. Though this mutation has been described as dominant negative in humans and mice, our data suggests that in flies this mutation is a hypomorphic allele of dFXR. To clarify this discrepancy, transgenic flies carrying the wild type and mutant mouse proteins are being generated.

Identification of Candidate Genes for Proteus Syndrome by Microarray Analysis. *S.J. Vacha¹, G. Gooden¹, K. Peters¹, M.M. Cohen, Jr.², P. Meltzer¹, L.G. Biesecker¹.* 1) NHGRI/NIH, Bethesda, MD; 2) Dalhousie University, Halifax, Nova Scotia, Canada.

Proteus syndrome (PS) is a rare, sporadic, overgrowth disorder for which there is no molecular data. Affected individuals present a mosaic distribution of lesions involving bone, adipose, cartilage, and vascular tissues. It is hypothesized that PS is due to a post-zygotic somatic mutation that is lethal in the non-mosaic state. While the sporadic occurrence of PS precludes a positional cloning strategy to identify the causative gene(s), the mosaic pattern of lesions within individuals allows for a molecular comparison of paired-tissue samples. Preliminary genomic experiments (comparative genomic hybridization, microsatellite analysis, representational difference analysis) did not detect aberrations between normal and affected tissues from within PS patients. For this reason, microarray analysis was performed to characterize gene expression differences between paired fibroblasts of three affected patients. RNA from these tissues was alternately labeled with Cy3 or Cy5 and hybridized to a 6K array. Green/Red hybridization ratios were corrected using 88 internal control genes and a 99% confidence interval was set. Of the 6,264 cDNAs, 17 target outliers were common to all three patient comparisons. 60 additional targets showed common patterns of expression, albeit below the established threshold. The identified target outliers have known roles in growth, signaling, and collagen deposition consistent with the neoplastic features of this disorder. By characterizing gene expression differences between mosaic lesions, we have identified candidate genes for the growth perturbation in PS.

PTEN mutations in Proteus and Proteus-like syndromes. X.P. Zhou¹, H. Hampel¹, R. Hennekam², J. Mulliken³, R. Winter⁴, C. Eng¹. 1) Human Cancer Genetics, Ohio State Univ, Columbus; 2) Univ Amsterdam, Holland; 3) Childrens Hosp, Boston; 4) Inst Child Health, London, UK.

Germline PTEN mutations cause Cowden syndrome (CS) and Bannayan-Riley-Ruvalcaba syndrome (BRR), 2 hamartoma-tumor syndromes. Because CS and BRR have some clinical overlap, and recent formal demonstration of overlapping PTEN mutation spectra, it was proposed that CS and BRR belong to a single genetic entity, PTEN-Hamartoma Tumor Syndrome (PHTS). In order to determine whether other syndromes of overgrowth and hamartomas are part of PHTS, we ascertained 7 with Proteus syndrome (PS) and 3 undefined Proteus-like (PL) cases who had the minimal features of lipomas, any hamartoma and overgrowth, but who did not meet the diagnostic criteria of CS, BRR, or PS and subjected them to PTEN mutation analysis. We identified germline PTEN mutations in 1 (33%) PL, R335X, and in 1 (13%) PS, W111R. While the PTEN mutation positive PS was classic by the Consensus Criteria, PL had features reminiscent of PS, CS and BRR but did not fit any single diagnosis. The PL was born with hypertrophy of the right lower extremity and macrocephaly. He developed massive arteriovenous malformations (AVM) in the muscles and bones of the right lower abdomen and lower extremity, lipomas and epidermoid nevi. A lipomatous mass, epidermoid nevus and AVM all harbored second hit mutations (R130X) on the allele opposite the germline R335X. The parents were PTEN wild-type. Both mutations have been described in CS and BRR. We postulate that the second hit, R130X, occurred early in embryonic development and may represent germline mosaicism. Both mutations affect phosphatase activity as well as truncate within or before the C-terminal C2 domain, which is important for phospholipid membrane binding. The second hit, R130X, on top of the first would result in PTEN null status in the affected tissues. Consequently, progressive and severe neoplastic-like overgrowth is the phenotype in those tissues which completely lack PTEN. Our observations suggest that a subset of PL and even PS can be associated with pathogenic germline PTEN mutations and are part of PHTS, with its implications for cancer risk and future surveillance.

Uniparental disomy testing in a clinical setting. *C.R. Pereira, D.L. Kennedy, E. Mak-Tam, A. Rezaie, M. Skuterud, J. Steer, J.P. Tzountzouris, D.J. Allingham-Hawkins.* Genetics, North York General Hospital, Toronto, Ontario, Canada.

Uniparental disomy (UPD) occurs when both copies of a homologous chromosome pair are inherited from the same parent with no contribution from the other parent. UPD of some chromosomes (i.e. 7, 11, 14 and 15) has been associated with a specific phenotype while, for other chromosomes (i.e. 13, 21 and 22), it appears benign. However, the data are limited in this emerging field. Our lab offers UPD testing for chromosomes 7, 13, 14, 15, 21 and 22 for the following indications: familial or de novo robertsonian translocation, reciprocal translocations, marker chromosomes, abnormal phenotype and mosaicism. To date, 54 cases have been analysed and 6 positives have been identified (11%). These positives include UPD14 in a child with a t(13;14), UPD15 in a child with an inv dup(15) and UPD15 in 3 children and 1 adult with clinical symptoms of Prader-Willi Syndrome (PWS). The 54 cases studied include 39 cases of familial or de novo robertsonian translocation, 33 of which were analysed prenatally. The most common robertsonian translocation was t(13;14) (22 cases) followed by t(14;15) and t(14;21) (4 cases each). Other reasons for referral included 4 cases with an inv dup(15), 4 cases of suspected PWS, 2 reciprocal translocations involving chromosome 14, 3 suspected cases of UPD7 (all negative) and 2 cases of mosaicism for trisomy 14 on prenatal samples. Our data suggest that routine UPD testing is warranted and informative under specific guidelines.

Analysis of a translocation breakpoint locus at 7q31 from an autistic individual reveals a complex multigene system. *J.B. Vincent, E. Petek, S.W. Scherer.* Department of Genetics, The Hospital for Sick Children, Toronto, ON., Ontario, Canada.

In a study aimed at identifying candidate genes for autism within regions implicated by linkage studies, we have recently cloned a novel gene, RAY1 (or FAM4A3), that spans a balanced translocation breakpoint (t(7;13)(q31.3;q21)) in an autistic individual. RAY1 contains 16 exons, with alternative splicing and use of two different 3'-terminal exons, encoding a protein from 554 to 585 amino acids in length, and spanning approximately 220 kb of genomic DNA at 7q31.3. The translocation breakpoint localizes between exons 9 and 10. In addition to RAY1 we have identified two apparent anti-sense genes, with no obvious continuous open reading frames. One of these also exhibits multiple isoforms due to alternative splicing and use of different polyadenylation signals. These anti-sense genes may function at the RNA-level, acting within the nucleus by controlling expression of sense gene RAY1. A fourth gene has also been identified which is transcribed from the same strand as RAY1, and encompasses RAY1 exons 11, 12 and 13, and at least 6 new exons. This gene also shows no continuous open reading frame. Although the 5'-ends of these three new genes have yet to be identified, it is likely that the translocation in the autistic individual disrupts several of these transcripts. Expression studies using northern hybridization and RT-PCR analysis show that, whilst RAY1 is ubiquitously expressed, expression of the two anti-sense transcripts is limited to just a few tissues, including testis, cerebellum and fetal brain. In order to determine whether any of these transcripts have a role in susceptibility towards autism, we are currently screening for mutations in them amongst probands from 95 multiplex autism families from the Autism Genetic Resource Exchange (AGRE), and have so far identified several DNA sequence variations. Further studies will be required to ascertain whether these changes represent mutations involved in the etiology of autism.

Mutation detection of LHON in Iranian families. *S. Miri Lavasani*^{1,2}, *N. Bazazzadegan*¹, *A. Tabasi*³, *E. Elahi*², *M. Houshmand*¹. 1) Medical genetic unit, National Research Center for Genetic Engineering and Biotechnology, Tehran, Iran; 2) Faculty of science, Tehran University, Tehran - Iran; 3) Neuroophthalmology unit, Farabi Hospital, Tehran Iran.

Human mitochondrial DNA (mtDNA) is a circular molecule of 16569 base pairs, which codes for 13 proteins of the respiratory chain enzyme complexes, 22 tRNAs, and 2 rRNAs. There are thousands of copies of mtDNA in a cell and a mutation can affect all (homoplasmy) or a fraction of the mtDNA molecules (heteroplasmy). The mtDNA is maternally inherited, i.e. only the mother transmits mtDNA to the children. Children of the same sib ship can inherit different proportions of normal and mutated mtDNA if the mother is heteroplasmic. mtDNA mutations cause different type of diseases such as Lebers hereditary optic neuropathy (LHON). LHON has traditionally been considered a disease causing severe and permanent visual loss in young adults (20-30 years old). One of this three pathogenic (primary) mitochondrial DNA (mtDNA) mutations (at position 11778 or 3460 or 14484) has been associated in nearly all families with LHON. In this first investigation in Iran, We have investigated Four point mutations (11778, 3460, 14484 & 14459) on 20 patients with LHON. The 11778 mutation has been identified in two Iranian families by using PCR and RFLP methods. The first patient was a 29 years old man. He has lost his vision progressively from the age 25. His older brother (38 years old) with complete visual loss was carrying the same point mutation. The symptoms of his disease started from the age 25. The mutation was homoplasmic. The second patient was a 25 years old man. He has lost his vision progressively from the age 21. He is carrying the same mutation. He has four brothers and four sisters. Two younger brothers (21 and 16 years old) have shown the same mutation. The mutation was homoplasmic. The investigation was performed for patient's sisters and his cousins but none showed any mutation. More investigations of inheritance of this mutation continue in this center.

Neonatal diabetes mellitus, occurrence of developmental abnormalities, genetic and epigenetic determinism. *E. Diatloff-Zito¹, E. Diaz¹, I. Le Monnier de Gouville³, J.J. Robert², C. Bouvattier⁴, P. Bougnres⁴, J. Hors³, C. Junien¹, C. Diatloff-Zito¹.* 1) INSERM U383 Groupe Hospitalier Necker Enfants Malades, Universit de Paris V, Paris, France; 2) Hpital Necker Enfants Malades, Paris, France; 3) Hpital Saint Louis, Paris, France; 4) Hpital Saint Vincent de Paul, Paris, France.

Neonatal Diabetes Mellitus (NDM) is a rare entity with an estimated incidence of 1/400 000 in Europe. Hyperglycemia occurring in the first six weeks of life, intrauterine growth retardation, low adiposity at birth are hallmarks of NDM. Transient (T) or permanent (P) NDM are initially indistinguishable. Though, (T)NDM resolves within 6 months, type 2 diabetes often reappears later in life. The genetic and molecular mechanisms of (P)NDM are poorly known. Chromosome 6 abnormalities with paternal isodisomy may occur in some (T)NDM, a candidate region for an imprinted gene to 6q24 is identified. The allelotype for chromosome 6 microsatellites markers was achieved in 15 NDM patients and relatives. Two patients out of 9 with a (T)NDM had a paternal chromosome 6 isodisomy. The spectrum of clinical features is suggestive of a gradient of epigenetic and/or gene mutation types. Various congenital abnormalities found in (P) and (T)NDM patients may be consequent to altered early developmental pathway(s). The study of candidate genes is under way. The genetic and molecular characterization of NDM may contribute to the elucidation of central junction of multiple signaling pathways leading to NDM, with a critical role in fetal development.

Genetic Analysis of Candidate genes in Nonsyndromic Cleft Lip with or without Cleft Palate Families. L.

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Nonsyndromic cleft lip with or without palate (NSCL/P) is a common genetically, complex birth defect, with prevalence from 1/500 to 1/1000 live births. Evidence from linkage, linkage disequilibrium and mouse models studies is contradictory suggesting that heterogeneity between study populations may exist. Previously, we reported significance evidence for heterogeneity at the TGFA locus in the Colombian families with and HLOD of 1.1 and 35 percent of the families linked by performing linkage analysis of candidate genes in 43 NSCL/P families from Colombia and 12 families from Columbus, Ohio. In addition, we reported the exclusion of TGFB3, MSX1 and the 6p23-25 region as candidate genes for NSCL/P in the Colombian families. A recent report of a genome wide scan in 92 sib pairs from the United Kingdom revealed suggestive linkage to 10 loci (Prescott et al. 2000). The purpose of this study is to replicate these results in the Colombian and Ohio families, which represent two distinct Caucasian populations, using parametric (dominant and recessive models) and nonparametric (GENEHUNTERnpl and SimIBD) linkage approaches. In addition, heterogeneity was analyzed using GENEHUNTER. Preliminary results showed no evidence of linkage to 1p36, 8q23-25, 11, Xcen-q21. Efforts are ongoing to recruit additional families and genotype more densely spaced markers for these loci and additional candidate genes to determine whether they have a role in CL/P in these populations.

Russell-Silver syndrome as a phenotype resulting from defects in the IGF1R axis: the ligand IGF2, its receptor IGF1R, or the signaling modulator GRB10. K. Kosaki¹, T. Kikuchi², H. Yoshihashi¹, R. Kosaki^{1,3}, T. Suzuki¹, R. Smith⁴, N. Matsuo¹. 1) Department Pediatrics, Keio University, School of Medicine, Tokyo, Japan; 2) Department Pediatrics, Niigata University, School of Medicine, Niigata, Japan; 3) Health Center, Keio University, Tokyo, Japan; 4) Joslin Diabetes Center, Harvard Medical School, Boston, MA.

Russell-Silver syndrome [RSS] is characterized by prenatal and postnatal growth retardation accompanied with dysmorphic features such as triangular facies and fifth finger clinodactyly. Patients with 15q26-qter deletions who are hemizygous for the *Insulin-like growth factor 1 receptor (IGF1R)* are known to present with features compatible with the diagnosis of RSS. Meanwhile, documentation of maternal uniparental disomy of chromosome 7 in 10% of patients with RSS has suggested the presence of an imprinted gene on chromosome 7 whose mutation is responsible for the RSS phenotype. Recently other groups reported two patients with RSS-like phenotype who had a maternally-derived duplication of 7p11-p13 encompassing *GRB10* (*Growth factor receptor-bound protein 10*), which regulates IGF1R-mediated growth and mitogenesis. We demonstrated that human *GRB10* is a maternally expressed imprinted gene and identified two patients with a maternally derived P95S substitution within the N-terminal proline-rich domain of *GRB10* by screening 58 RSS patients. We further documented that a patient with a maternally-derived duplication of 11p [46,XX,-16,+der(16)t(p15;q24)], who presented with RSS-like phenotype, has duplications in *IGF2* and *H19*. Considering that *IGF2* is a paternally expressed growth promoting gene whereas *H19* is a maternally expressed growth suppressing gene, growth retardation in maternal duplication of 11p could be due to relative suppression of IGF2 expression. It is noteworthy that paternally derived duplication of 11p15 is associated with overgrowth (i.e. Beckwith syndrome) as opposed to growth retardation. These findings collectively give credence to the concept that RSS represents a common phenotype caused by defects in any of several proteins in the IGF1 receptor axis: the ligand IGF2, its receptor IGF1R, and the IGF signaling modulator GRB10.

The dopamine system genes and the risk for substance abuse. *M.M. Vanyukov^{1,2,3}, B.S. Maher², H.F. Simkevitz¹, L. Kirisci¹, R.E. Ferrell², M.L. Marazita^{2,4}, G.P. Kirillova¹, R.E. Tarter^{1,2}.* 1) Dept. of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, PA; 2) Dept. of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 3) Dept. of Psychiatry, University of Pittsburgh, Pittsburgh, PA; 4) School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA.

Liability to substance dependence (SD) is a multifactorially inherited trait whose variation in the population is significantly contributed by genotypic differences. The heritability of SD liability is based on polymorphisms at the genes that are likely to be related to the function of the central nervous system and, possibly, the biotransformation of xenobiotics. We have recently shown the dopamine D5 receptor gene's (DRD5) associations with liability to SD in Caucasian males and females. We report a replication of this association, for categorical diagnosis and for an index of severity of substance-use-related problems, in a case-control study in an independent sample of Caucasian males from the same geographic region in the US. In addition, a marginally significant association of the risk for SD with a microsatellite polymorphism in the dopamine D2 receptor gene was observed. No gene-gene interaction effect between these genes representing the two dopamine receptor families was detected. The data obtained are consistent with the contribution of genetic variation in the dopamine system to individual differences in substance dependence liability.

Aberrant splicing of the insulin receptor pre-mRNA could contribute to insulin resistance in the trinucleotide repeat disorder, Myotonic Dystrophy. *R.S. Savkur, A.V. Philips, T.A. Cooper.* Pathology, Baylor College of Medicine, Houston, TX.

Myotonic Dystrophy (DM1) is an autosomal dominant, multisystemic disorder caused by the expansion of an unstable CTG trinucleotide repeat in the 3' untranslated region of the myotonic dystrophy protein kinase (DMPK) gene. In addition to the myotonia and muscle weakness that are the hallmarks of DM1, these patients also exhibit increased insulin resistance. The mature insulin receptor (IR) exists as two isoforms, A and B, which result from alternative splicing of the α -subunit pre-mRNA. The A isoform, which lacks exon 11, is less sensitive to the effects of insulin. RT-PCR analysis of RNA isolated from skeletal muscle and heart tissues of normal and DM1 individuals indicates that the primary IR transcript undergoes an aberrant switch in splicing, such that exon 11, which is normally included, is excluded in DM1 patients. Interestingly, fibroblast cells cultured from normal and DM1 individuals replicate the switch to the excluded form only when transformed with a MyoD-expressing retroviral vector and differentiated into skeletal muscle. Western blot analyses of skeletal muscle cultures and tissues indicate that the steady-state expression levels of CUG-Binding Protein (CUG-BP), the hnRNP protein implicated in DM1 pathogenesis, are elevated in the disease. Co-transfection of either an expression plasmid for CUG-BP, or a DMPK minigene containing expanded CTG repeats, with the IR gene in mouse C2C12 myoblasts also replicates the switch in the isoform. These results demonstrate that aberrant splicing of the IR in DM1 is observed in those tissues that are sensitive to insulin and primarily affected in the disease. Our results also suggest that altered expression of CUG-BP as a result of expanded CUG-RNA could cause aberrant splicing of the IR, thus rendering DM1 patients resistant to insulin.

Aberrant pre-mRNA alternative splicing in Myotonic Dystrophy: Implications for disease phenotype. A.V.

Philips, R.S. Savkur, T.A. Cooper. Pathology, Baylor College of Medicine, Houston, TX.

Myotonic Dystrophy (DM 1) is an autosomal dominant, multisystemic disorder caused by a CTG trinucleotide repeat expansion in the 3' untranslated region of the DM protein kinase gene. Symptoms include myotonia, cardiac conduction defects, and endocrinopathy. One model for this disease proposes that RNA from the expanded allele causes a gain-of-function mutation that disrupts the processing or transport of heterologous RNA by binding or altering the function of proteins involved in RNA processing. We report that alternative splicing of a number of genes is disrupted in DM 1 patient tissues, notably heart, skeletal muscle and brain. These include the insulin receptor (IR), ryanodine receptor, NMDA, amyloid precursor protein, and tau. Of particular interest is the aberrant splicing of IR because of the documented insulin resistance in DM 1 patients. IR exists as two isoforms that include (isoform B) or exclude (isoform A) exon 11 of the α subunit. Isoform A has a higher binding affinity for insulin while B is believed to have a more efficient signaling response to insulin. The B isoform is expressed mainly in insulin sensitive tissues and comprises 60-70% of the IR mRNA in normal skeletal muscle and heart. In DM 1 tissues B isoform levels decrease to 10-20% of IR mRNA in skeletal muscle and to 40% in heart. We replicated this switch in isoform ratios in a transient transfection system using IR minigenes in C2C12 mouse myoblasts. Cotransfection of IR minigenes with 1440 CTG repeats resulted in a decrease in B isoform levels indicating that the expanded CTG repeats probably cause the switch in isoform ratios in DM 1 tissues. Similar results were observed by cotransfections of IR minigenes with an expression plasmid for ETR-3, a homolog of CUG-binding protein (CUGBP), indicating that ETR-3 or a member of this protein family might be the mediator of the disrupted splicing in DM 1 tissues. Aberrant splicing of the IR was duplicated in DM 1 fibroblasts which were transformed into myoblasts and differentiated. We speculate that these changes in the insulin receptor isoform ratios could be the basis for the insulin resistance manifested in DM patients.

CONGENITAL HYPOTHYROIDISM: A POPULATION BASED STUDY. V. Tassi¹, F. Calaciura², G. Miscio¹, A. Majorana², G. Parrinello², A. Ranieri¹, C. Regalbuto², L. Sava², V. Trischitta¹. 1) Research Unit of Endocrinology, IRCCS-CSS, S.Giovanni Rot., Foggia, Italy; 2) Ist. Internal Medicine, Endocr. & Metab. Diseases, Univ. Catania, Italy.

Introduction Congenital hypothyroidism (CH) is a frequent condition that, if not precociously recognized, may lead to severe neurologic and developmental disturbances. CH can be characterized by a variable degree of thyroid development failure (dysgenesis) or by thyroid enlargement (goiter), mostly due to inherited defects in thyroid hormone biosynthetic pathway. Several genetic abnormalities may play a role in CH, but their actual prevalence is still unknown since population based study have never been performed so far. **Purpose** of the present report is the study of two different CH phenotypes, namely CH with goiter and CH with normal/hypoplastic thyroid, investigated by the analysis of three candidate genes. **Methods.** CH patients were identified in Eastern Sicily in the course of routine screening for CH. Newborns having TSH at day 3-6 higher than 20 mU/ml of blood were recalled and the diagnosis confirmed in 59 newborns on the basis of serum TSH and T4 measurements. Out of them we studied 10 newborns affected by CH with goiter and 8 with normal/hypoplastic thyroid. Candidate genes were analyzed on genomic DNA by SSCP and direct sequencing. Thyroperoxidase gene, playing a key role in thyroid hormone synthesis, was analyzed in the 10 CH patients with goiter and Thyrotropin receptor and Pax 8 gene, playing important roles in thyroid differentiation and development, were analyzed in the 8 CH patients with normal/hypoplastic thyroid. **Results:** in CH patients with goiter 3 patients carried mutations of the TPO gene. One patient was a double heterozygous carrying a 1277^{ins} GGCC and a 1996^{G→T} Glu→Stop mutation. Two patients were heterozygous, carrying, respectively, a 1277^{ins} GGCC and 2295 G→A mutation inducing a Val→Ile change. No mutation was identified in the 8 CH patients without goiter. In **conclusion**, our study represent so far the first population based approach to CH and might contribute, through a better phenotypic characterization, to a more useful classification of CH.

Polycystic Ovary Syndrome (PCOS): a family study of phenotypic expression and candidate genes. E.B.

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Polycystic ovary syndrome (PCOS), a heterogeneous disorder characterized by chronic anovulation and hyperandrogenism, affects 5-10% of women of reproductive age. Affected women have an increased risk to develop impaired glucose tolerance, gestational diabetes mellitus, and type 2 diabetes mellitus. Although the familial nature of this disorder has been recognized and despite efforts of many investigators, the PCOS genes remain elusive. To determine whether phenotypic heterogeneity occurs within families and to examine several candidate genes, we identified four families in which there were multiple affected females. The proband was a female child with premature pubic hair (n=1) or an adolescent girl with hirsutism and/or oligomenorrhea (n=3). A detailed family history was obtained from the proband's mother. Medical history questionnaires were completed for each participating family member together with collection of blood samples for hormone determinations (androstenedione, testosterone, free testosterone) and candidate gene analyses (CYP21, HSD3B2, IRS-1, GRL, ADRB3, CYP19, CYP17, AR). Phenotypic variability was noted to occur both between and within each family. In one family, the PCOS phenotype appeared to be linked to the G972R variant of the insulin receptor substrate-1 (IRS-1) gene and possibly CYP21. A variant of the 3'-UTR of the 3-beta-hydroxysteroid dehydrogenase 2 (HSD3B2) gene was identified in the proband of a second family. In these four families, no linkage was apparent for CYP19, GRL, or AR, and linkage to CYP17 was significantly excluded. The extreme phenotypic heterogeneity even within single families complicates traditional genetic analyses of this disorder. With identification of genetic markers, it may become possible to sub-classify affected individuals to predict risk for consequences and select the most appropriate therapeutic intervention(s).

A candidate gene responsible for transient neonatal diabetes. *Y. Makita^{1,2}, J. Inoue³, K. Mitsuya³, K. Imada⁴, T. Honma⁵, Y. Ito², R. Mitamura², T. Ishii², M. Oshimura³, A. Hata².* 1) Public Health, Asahikawa Medical College, Asahikawa, Hokkaido, Japan; 2) Pediatrics, Asahikawa Medical College, Asahikawa, Hokkaido, Japan; 3) Molecular and Cell Genetics, School of Life Science, Faculty of Medicine, Tottori University, Yonago, Tottori, Japan; 4) Pediatrics, Niigata Prefectural Sakamachi Hospital, Sakamachi, Niigata, Japan; 5) Pediatrics, Niigata City General Hospital, Niigata, Niigata, Japan.

Transient neonatal diabetes mellitus (TNDM), a rare form of childhood diabetes (an incidence of ~1 in 400,000 live births), presents in growth retarded neonates with persistent hyperglycemia. Recovery is usually complete by 18 months of age, however 40% of the patients relapse and develop diabetes again later in life. The association of both paternal uniparental disomy of chromosome 6 (UPD 6) and paternal duplications of 6q24 with TNDM suggested the mechanism of the disease to be overexpression of an imprinted gene in this locus. With an analysis of TNDM patients, the responsible gene is revealed to reside 461 kb region of PAC contig (CEN-dJ210B1-dJ468K18-dJ340H11-dJ197L1). In this region, we found two paternally expressed genes by the method with mouse human hybrid cell lines. One has been reported by the name *PLAGL1/ZAC*, and the other is newly identified gene, tentatively named X. Total 10 Japanese TNDM patients and their relatives were analyzed with newly developed four microsatellite markers located nearby the two genes to see UPD or duplication. In two out of ten patients, paternal duplication exists throughout the region where present markers locate. However, in one family, only one marker locating centromere side of dJ197L1 is found to be duplicated. Combined with the fact that *PLAGL1/ZAC* gene is known to be involved in the PAC clones named dJ468K18 and dJ340H11 strongly indicates that the X is a responsible gene for TNDM. Analysis of methylation status of gene X in the remaining 4 patients without any UPD or duplication is now ongoing.

Question of switched babies in two hospitals: two unique genetic mechanisms and not misidentification in the nursery. *L.R. Shapiro*^{1,2}, *R.V. Lebo*³, *S.J. Brown*⁴, *J.M. Hoover*², *D.F. Kronn*^{1,2}. 1) Pediatrics/Medical Genetics, New York Medical Col, Valhalla, NY; 2) Westchester Medical Center, Valhalla, NY; 3) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 4) Columbia University College of Physicians and Surgeons, New York, NY.

A 3 9/12 year old African American girl has no hemoglobin C on electrophoresis (A=91.2%; F=3.5%; A₂=5.3%), but her mother has Hemoglobin C Disease (CC). Because of elevated Hemoglobin A₂ and microcytosis (MCV=59), β Thalassemia Minor was diagnosed in the child. Hemoglobin electrophoresis of the mother was 100% hemoglobin C. The possibility of non-maternity was considered because the mother had pre-eclampsia and seizures at delivery and was not able to see her baby for 3 days. After confirming all results for mother and child, DNA testing revealed 99.95% probability of maternity. Amplification of the β globin gene revealed 2 peaks representing Hemoglobin C and β^0 Thal which was confirmed by gene sequencing. Thus, the mother is C/ β^0 Thal and the child is A/ β^0 Thal, receiving the β^0 Thal from her mother.

DNA sequence analyses for an affected child with Maple Syrup Urine Disease (MSUD) and his parents were done in preparation for prenatal diagnosis of a second pregnancy. The affected child was found to be homozygous for a deletion of the E2 gene which rendered it nonfunctional. The fetus was unaffected and not heterozygous. Mutation analyses of the parents were not reported until the next (third) pregnancy, and no somatic mutations for MSUD were found. All testing was repeated and all results confirmed. DNA testing using highly polymorphic markers for both parents definitively proved maternity and paternity. The third fetus was also unaffected and not a carrier. Utilizing 16 probes syntenic to the MSUD gene on the maternal chromosome 1, a previously undescribed mechanism was delineated which indicated how a homozygous affected offspring arose from non-carrier parents: maternal de novo mutation prior to maternal meiosis I ® nondisjunction in maternal meiosis II ® fertilization ® trisomy 1 ® rescue ® uniparental disomy 1 with homozygosity for the MSUD gene.

Systematic search of molecular variants of the human synapsin 3 gene and association study with schizophrenia.

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Human synapsin 3 gene is a newly identified member of synapsin gene family with putative function of regulating synaptogenesis and neurotransmitter release. The gene was mapped to 22q12-13, a possible region that may harbor schizophrenia gene as suggested by several linkage studies. Hence, the synapsin 3 gene was considered a candidate gene of schizophrenia. We systematically searched for mutations in the protein coding and 5'-promoter regions of the synapsin 3 gene in a sample of Chinese schizophrenic patients from Taiwan. Three single nucleotide polymorphisms were identified: g.-631C>G and g.-196G>A at 5-end promoter region, and g.69G>A at exon 1. Further case-control association studies, however, did not find significant differences of genotype or allele frequency distributions of these three polymorphisms between 163 patients and 151 non-psychotic comparisons. Hence, we suggest that the human synapsin 3 gene may not contribute substantially to the pathogenesis of schizophrenia.

Somatic instability of CAG repeat occurs in the HD gene knock-in mouse. *H. Ishiguro¹, K. Yamada¹, H. Sawada¹, K. Nishii¹, N. Ichino¹, M. Sawada¹, Y. Kurosawa¹, N. Matsushita², K. Kobayashi², J. Goto³, I. Kanazawa³, T. Nagatsu¹.*
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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by involuntary movement, personality change, and dementia. The underlying genetic alteration is a CAG repeat expansion in exon 1 of the HD gene, and a clear relation exists between CAG repeat length and age of onset. Instability of CAG repeat length through meiotic transmission in males is considered to be implicated in the expansion of the triplet repeats, but the mechanisms of somatic CAG repeat instability are still unclear. We have generated HD gene knock-in mice in which the exon 1 of the mouse HD gene was replaced by the human exon 1 containing 77CAG repeats. Wild and mutated huntingtin proteins were found to be expressed ubiquitously in brain and peripheral tissues. To determine the number of CAG repeats, Genescan analysis was performed using genomic DNA prepared from various tissues. A mouse identified with 97 CAG repeats in exon 1 of HD gene was found to be mated with homozygous (77 CAG repeat, male) and female normal mice. Although this is rare case of the CAG expansion through meiotic transmission, it could be a similar situation in the human case. In addition, a one or two CAG repeat expansion was found in 25% of the litters from paternal transmission. The CAG repeat in 20% litters, by contrast, was contracted through maternal transmission. Expanded CAG repeat instability (somatic CAG mosaicism) in 75 week-old mice was found in brain except for the cerebellum, and in peripheral organs such as the liver, kidney and stomach. We confirmed the same results of expanded CAG repeat instability in human brain of HD patients. The CAG repeat instability of this mouse model may mirror the abnormalities in HD patients.

AGG Interruptions in the CGG Trinucleotide Repeat Tract of the FMR1 Gene May Contribute to Stability of Fragile X Premutations. *S. Dyack*¹, *L. Steele*³, *G. Koulchitski*³, *Y. Yang*³, *R. Weksberg*¹, *P.N. Ray*^{2,3}, *C.E. Pearson*². 1) Division of Clinical and Metabolic Genetics; 2) Genetics and Genomic Biology; 3) Department of Pediatric Laboratory Medicine, The Hospital for Sick Children, The University of Toronto, Toronto, Ontario, Canada.

Fragile X Syndrome (FRAXA), the most common form of inherited mental retardation (incidence 1/2000-1/4000), is associated with expansion of CGG triplet repeats in the FMR1 gene. The number of CGG trinucleotide repeats in the normal population is variable, ranging from 6-60. Affected individuals have greater than 200 CGG repeats. CGG trinucleotide repeats in the range of 52-200 may exhibit meiotic instability and are referred to as premutations. Small premutations may expand by several repeats during meiosis but rarely expand to full mutations in the next generation. Factors thought to contribute to the stability of premutations include the presence of AGG interruptions within the CGG repeat tract as well as the length of pure CGG repeats. We have investigated two unusual families, in which a small premutation found in a carrier female expanded to a full mutation in only one generation. One female with 66 CGG repeats had an affected son with 630 CGG repeats, while a second female with 58 CGG repeats had an affected son with a mosaic pattern of 100, 230, and 600 repeats. We compared these two ascertained females to a control female who had a FRAXA allele with 59 CGG repeats, which had been transmitted without expansion to the next generation. After PCR amplification of the CGG trinucleotide repeat tract, the normal and premutation alleles were electrophoretically separated and sequenced. The two females with premutation alleles that expanded in the next generation to a full mutation had no AGG interruptions whereas the premutation allele of the control that had been transmitted without expansion to the next generation had two AGG interruptions. We speculate that the lack of AGG interruptions has contributed to the surprising level of instability exhibited by these premutation alleles.

"Mitotic Drive" of Expanded CTG Repeats in Myotonic Dystrophy Type 1 (DM1). *M. Khajavi*^{1,2}, *A.M. Tari*³, *N.B. Patel*³, *K. Tsuji*^{1,2}, *M.L. Meistrich*³, *N.H.A Terry*³, *T. Ashizawa*^{1,2}. 1) Baylor College of Medicine, Houston, TX; 2) Veteran Affairs Medical Center, Houston, TX; 3) The University of Texas MD Anderson Cancer Center, Houston, TX.

The expanded CTG repeat in myotonic dystrophy type 1 (DM1) shows age- and tissue-dependent somatic instability with a bias toward further expansion, accounting for anticipation, phenotypic variability, and perhaps, progressive disease course. Various models have been proposed to explain this expansion bias based on unusual DNA structures formed by the CTG repeat tract that give rise to an inherent tendency for expansion. Here, we present evidence for a novel mechanism that can explain the expansion bias observed in peripheral blood leukocytes, independent of the DNA structure-based models, but on the basis of preferential proliferation of cells with larger CTG repeats.

We established 22 clonal lymphoblastoid cell lines (LBCLs) from 18 DM1 patients by single-cell cloning. Six of these 22 cell lines yielded a rapidly proliferating mutant cell population that showed a gain of large CTG repeats (by 40 - 290 repeats). The mutant cell population continued to grow and eventually replaced the progenitor population. From such clonal lines, we subcloned additional cell lines carrying different sizes of the expanded CTG repeat with identical genomic background. Mixing these subclones in pairs ($n = 6$) in culture consistently resulted in preferential survival of subclones with larger expanded repeats over 3-10 passages. In these subclones, repeat size showed a strong correlation ($r^2=0.84$) with cell proliferation rate and Erk1,2 activities, and an inverse correlation with the p21^{WAF1} level. Size of the CTG repeat expansion showed no correlation with Akt activity or levels of Erk1/2, p53, Bcl-2 or Bax in DM1 LBCL subclones. Our data suggest that Erk1,2/p21^{WAF1}-mediated growth advantage of cells with larger CTG repeats (hence, "mitotic drive") is a strong driving force for further CTG expansion at the tissue level, which explains the observed expansion bias independent of the DNA-based models.

Autism LOD on chromosome 7q was increased subsetting the sample on language acquisition. *S.E. Folstein¹, for CLSA^{2,3,4}.* 1) Dept Psychiatry, Box 1007, New England Medical Ctr, Boston, MA; 2) Department of Psychiatry, University of North Carolina Chapel Hill, NC; 3) Department of Genetics Vanderbilt University Nashville, TN; 4) Department of Human Genetics University of Iowa Iowa City, IA.

In the CLSA genome screen of 75 families ascertained through two autistic siblings, our maximum 2-point heterogeneity LOD for chromosome 7q31-33 was 1.13 at D7S1813, 103.6 cM. A second smaller signal was seen distally at GATA32C12, 150 cM near CFTR. Signals in this region have been found for autism by several groups. Two separate studies of families with developmental language disorders have also reported linkage or association with the CFTR locus. Delayed development of speech and language is a frequent, although not universal, finding in autism. Speech and language problems are also frequent in parents and siblings of autistic children. We divided our 75 families into two groups, based on language acquisition in the probands. There were 50 families where both probands had onset of phrase speech after 3 years and 25 families where one or both probands had normal onset of speech. We additionally coded any parent as affected who reported either delayed onset of speech, trouble learning to read, or persistent spelling difficulties. Other parents were called unknown. The 2 point heterogeneity LOD for the 50 language-abnormal families was 2.77 at D7S1813, and the LOD at this marker for the 25 language-normal families was 0.00. Our findings suggest the hypothesis that autism and developmental language disorder, both thought to be inherited through an oligogenic mechanism, may have at least one gene in common.

FISH analysis of two translocations of chromosome 15q associated with dyslexia. *M. Taipale*¹, *J. Nopola-Hemmi*^{1,2}, *T. Haltia*³, *A.-E. Lehesjoki*¹, *A. Voutilainen*², *J. Kere*^{1,4}. 1) Department of Medical Genetics, Haartman Institute, University of Helsinki, Finland; 2) Department of Pediatric Neurology, Hospital for Children and Adolescents, University of Helsinki; 3) Department of Medical Chemistry, Institute of Biomedical Sciences, University of Helsinki; 4) Finnish Genome Center, University of Helsinki.

Specific reading disability, or developmental dyslexia, is characterized by unexpected difficulties in reading and spelling despite of adequate intelligence, education and social environment. It has a significant genetic component, and several independent studies have found predisposing loci in chromosomes 6p21 (*DYX2*) and 15q21 (*DYX1*). We have identified two Finnish families with balanced translocations involving chromosome 15q21-q22. In the first family, a translocation t(2;15)(q11;q21) segregates coincidentally with dyslexia. In the second family, a translocation t(2;15)(p13;q22) is associated with dyslexia in one family member. We have performed fluorescent *in situ* hybridisation (FISH) studies to further map the breakpoints in chromosome 15q. Both breakpoints map within the same 6-8 Mb, or 2,2 cM, interval in 15q21. This region is limited by markers D15S143 and D15S1029, and thus partly overlaps the *DYX1* locus implicated in previous linkage studies. Our results support the existence of at least one dyslexia locus in 15q21 and should aid in the identification of the genes involved in the development of dyslexia. The molecular cloning of both breakpoints is currently underway.

Association Analysis of GI Candidate Genes in Austistic Disorder. *M.P. Bass¹, M.M. Menold¹, K.L. Joyner¹, C.M. Wolpert¹, S.L. Donnelly¹, S.A. Ravan², C. McClain³, L. vonWendt⁴, J.R. Gilbert¹, H.H. Wright², R.K. Abramson², G.R. DeLong¹, M.L. Cuccaro², M.A. Pericak-Vance¹.* 1) Duke University Medical Center, Durham, NC; 2) W.S. Hall Psychiatric Institute, University of South Carolina, Columbia, SC; 3) University of New Mexico Health Sciences Center, Albuquerque, NM; 4) Helsinki University Central Hospital, Helsinki, Finland.

Our genomic screen for Autistic disorder (AD) identified a broad area of interest on chromosome 2q (Bass 1999) as harboring potential AD susceptibility genes. Patients with AD often experience gastrointestinal (GI) complaints, including chronic gastritis, diarrhea, and abdominal pain (Horvath 1999). Accordingly, two genes in this region merited investigation, the human secretin receptor (SCTR) in 2q14.1 and D2S116 in 2q33, which is associated with celiac disease (CD) (Holopainen 1999).

Secretin is a hormone that stimulates the pancreas to release enzymes, potassium ions and bicarbonate. Administration of secretin to some children with AD improves their GI symptoms as well as their social behavior, communication and language skills (Horvath 1998). Candidate gene analysis of SCTR was carried out using single strand conformation polymorphism (SSCP) of a single nucleotide polymorphism (SNP) located just beyond the gene. We tested for linkage disequilibrium (LD) in 91 multiple and 124 single incidence families using the pedigree disequilibrium test (PDT) ($p=0.38$). Additional SNPs have also been identified in exons 10 and 12, and genotyping is ongoing.

CD is a digestive disorder of the small intestine that is characterized by gluten sensitivity, chronic diarrhea, and pain after eating. Analysis for LD at this locus yielded a global p -value of 0.31, but a nominal allele-specific p -value of 0.007 for the 150 allele. Linkage analysis of this marker in our data set is positive, with a MLS of 0.96 in our 91 AD sibpair families. There was evidence for increased paternal sharing at this locus (72.2% v. 52.6%). These data provide additional evidence of involvement of chromosome 2 in the genetics of AD.

Racial and ethnic differences in genotype distribution of a marker within the inflammatory bowel disease susceptibility locus IBD2. *S.M.S. Uthoff, M.R. Eichenberger, L.E. Hunt, S. Galandiuk.* Department of Surgery, University of Louisville, Louisville, KY.

Inflammatory bowel disease (IBD) includes **Crohn's disease (CD)** and **ulcerative colitis (UC)**, which are autoimmune diseases affecting over one million Americans. It is believed that UC and CD are polygenic disorders sharing some but not all susceptibility loci. The highest evidence for linkage to IBD has been reported for a region of chromosome 12q14 surrounding D12S83 with a LOD score of 5.47 and a positive transmission disequilibrium test. Flanking microsatellites showed distortion of allele sharing with a confidence interval spanning 41cM and thus designation of this locus as **IBD2**. We aimed to confirm this putative IBD2 region by genotyping D12S1022, a highly polymorphic microsatellite with close proximity to D12S83 (www.marshmed.org/genetics). The cohort comprised 338 individuals including 93 non-related patients with UC, 139 with CD, and 106 population-based control subjects. All patients had a similar affection status and were from the same geographic area. D12S1022 was amplified with polymerase chain reaction and genotypes were determined by autoradiography.

Analyzing the data unmatched for race and ethnicity, they show a significant association of allele D with UC versus controls ($c^2=8.1$; $p=0.005$) and CD ($c^2=3.9$; $p=0.048$), and for genotype DD with UC versus CD ($c^2=4.1$; $p=0.043$). Interestingly, subgroup analysis revealed a striking difference in genotype distribution among African-American and Jewish patients with **Crohn's disease**, when compared with the affected Caucasian population: The frequency of genotype CD was 21% in Caucasian, but significantly more common with 83% in **Jewish** ($c^2=9.2$; $p=0.002$) and 50% in **African-American** ($c^2=3.0$; $p=0.08$) patients. Surprisingly, we did **not** find a significant association between D12S1022 and IBD within the **Caucasian** population by excluding just 16 patients. These data provide further evidence that there may be significant genetic heterogeneity between different ethnic and racial populations.

Refinement of a psoriasis locus at chromosome 17q24-q25 (PSORS2) with association mapping. *A.M. Bowcock¹, C. Helms¹, R.A. Speckman¹, A. Menter².* 1) Washington Univ. Med. Ctr., St Louis, MO 63117; 2) Texas Dermatology, Dallas, TX 75230.

Psoriasis is a complex genetic disease affecting ~ 1% of the Caucasian population. It results in hyperproliferation of keratinocytes and recruitment of T-cells to the dermis and epidermis. Epidermal hyperplasia, scaling and inflammation are the result. A genetic basis of psoriasis has been demonstrated with twin and family studies and a strong association with HLA class I alleles has been demonstrated. Alleles in strongest disequilibrium with psoriasis map close to HLA-C, with HLA-Cw*0602 being the most strongly associated HLA-C allele. We have examined 17 multiply affected families and 250 nuclear families for linkage/association with HLA, and ~ 40% of our multiply affected families and 250 nuclear families harbor HLA-Cw*0602 alleles in all affected members. However, we and others have demonstrated the existence of other causative loci that include chromosome 1q21 (PSORS4), 3q21, 4qter (PSORS3), 19p and 17q24-q25 (PSORS2). We first described linkage of psoriasis to chromosome 17q24-q25 in several multiply affected families that did not provide evidence for linkage or association with HLA. Although this linkage finding has been replicated in some other psoriasis linkage studies performed by other groups, we have failed to see strong evidence for linkage in our 250 nuclear families. However, genotyping of >20 polymorphic microsatellites from 17q24-q25 has revealed evidence for association with alleles at loci within a 300 kb interval (HRR $p < 0.0007$; case/control $p < 0.01$) in this family set. In order to identify the associated allele, DNA sequencing of exons from this interval predicted by GENSCAN is being performed on pooled samples of cases and controls. Currently, sequencing of 32 putative exons has revealed 12 common single nucleotide polymorphisms (SNPs) with a SNP frequency of 1/560bp. Two SNPs are at significantly higher frequency in psoriasis cases. The identification of alleles from this region that are in strong linkage disequilibrium with psoriasis should considerably reduce the number of candidate genes for PSORS2 and pave the way for the identification of a non-HLA psoriasis gene.

A new locus for Multiple sclerosis map at ch17q11.2 and a polymorphism found in the Oligodendrocyte-Myelin glycoprotein. *R. Raha-Chowdhury*¹, *J. Coadwell*², *P.C. Emson*², *J. Trowsdale*¹. 1) Dept of Pathology, University of Cambridge, Cambridge, UK., England; 2) Molecular and Cognitive Neuroscience, The Babraham Institute, Cambridge, UK.

Multiple sclerosis (MS) is a complex chronic neurological disease with a suspected autoimmune pathogenesis. One in every 1,000 people in Europe suffers from MS, yet little is known of the genetic and environmental factors that influence susceptibility. We investigated two patients with neurofibromatosis 1 (NF1) who both developed the primary progressive form of MS. The gene for NF 1 is located on chromosome 17q11.2 and spans about 300 kb. Three genes, oligodendrocyte-myelin glycoprotein (OMgp), EV12A and EV12B, are embedded in an intron of the NF1 gene on the opposite strand. The OMgp is a phosphatidylinositol-linked membrane glycoprotein expressed in the brain. It is a 120-kDa, heavily glycosylated polypeptide of central nervous system myelin and oligodendrocytes. It is anchored in the outer leaflet of the plasma membrane through a glycosylphosphatidyl inositol lipid molecule like the neural cell adhesion molecule. It is a minor glycoprotein of central nervous system myelin whose temporospatial expression in human development coincides closely with the caudal to rostral progression of central nervous system myelination. We have found a polymorphism in exon 2 of the OMgp gene. The frequency increase in MS patients over controls (Number of MS Patient = 52, Gene frequency 24%, Heterozygosity rate 0.48, Number of controls = 100, gene frequency 3%, heterozygosity rate 0.03). The OMgp has the potential to function as an adhesion molecule and a variation could disrupt the interaction between the plasma membranes of oligodendrocytes and axons required for myelination.

CCR5D32 mutation is not associated with asthma in Denmark and US Caucasian populations. *C.S Sprankle¹, G.P Sreekumar¹, C.G Binnie¹, E.G Carden¹, S. Sharma¹, Q.A Nguyen¹, S.R Sheff¹, M. Blumenthal², J. Vestbo³, J. Sundry⁴, M.A Pericak-Vance⁴, S. Brewster¹, M.G Ehm¹, D.K Burns¹, M.J Wagner¹.* 1) Glaxo Wellcome, Inc, Research Triangle Park, NC; 2) Department of Medicine, University of Minnesota Medical School, Minneapolis, MN; 3) Department of respiratory Medicine, University of Copenhagen, Denmark; 4) Duke University Medical Center, Durham, N.C.

CCR-5 is a cell surface receptor (chemokine receptor) expressed on monocytes and some CD4+ cells. It has been reported that individuals carrying the CCR5D32 mutation, a naturally occurring variant of CCR5 gene which generates a premature protein, have a reduced risk for developing asthma (Hall et al, Lancet 1999,354(9186):1264-5). We genotyped 89 Caucasian nuclear families with at least one offspring affected with asthma (physician's diagnosis) from Minnesota, 197 nuclear families from Denmark, and 200 cases and 100 controls from a US Caucasian sample from North Carolina using allele specific amplification with a TaqMan fluorogenic probe/a PCR size discrimination assay. We investigated the evidence for association with asthma using several phenotypes: diagnosis by a physician, strict asthma (2 or more symptoms and a positive methacholine challenge test or broncho-dilator test) and bronchial hyper-responsiveness (positive methacholine reaction at or below 10mg/ml of methacholine). The frequency of the mutant allele was 9.9% and 11.2% in Minnesota and Denmark populations respectively. The frequencies were 11.05 % in cases and 8.5 % in the controls in the case-control sample. We analysed the data using transmission disequilibrium test (TDT) for the family data and Fisher's exact test for the case control data. Our studies show that CCR5D32 mutation is not associated with asthma in the 3 Caucasian populations under study. Several studies have investigated the role of CCR5D32 mutation in diseases in which its ligands (RANTES, MIP 1a and MIP 1 b) may be involved and there are no diseases associated with the absence of functional CCR-5 protein, suggesting the CCR-5 receptor may be expendable.

Linkage of an ADHD phenotype to 6p21.3 in a population with reading disability. *S.D. Smith¹, B.F. Pennington², E. Willcutt³, K. Deffenbacher¹, D. Hoover¹, A. Smolen³, R. Moyzis⁴, R.K. Olson³, J.C. DeFries³.* 1) Munroe Meyer Inst, UNMC, Omaha, NE; 2) U. of Denver, Denver, CO; 3) Inst. for Behav. Genetics, U. of Colo., Boulder, CO; 4) U. of California, Irvine, CA.

Attention Deficit Hyperactivity Disorder (ADHD) and Reading Disability (dyslexia) are common childhood disorders, both of which have been shown to be influenced by separate genetic factors. Association analyses have shown significant association with alleles of the dopamine transporter DAT1 and the dopamine receptor DRD4, while linkage with dyslexia has been reported for several chromosomal regions, particularly 6p21.3. These conditions often co-occur in children, and could either be due to pleiotropic effects, behavioral phenocopies or independent loci. We are testing alternative explanations for this comorbidity through linkage analysis of candidate regions in sib pairs, at least one of whom has reading disability, and for whom ADHD phenotypes are available. Linkage of dyslexia to chromosome 6p21.3 was demonstrated in a subset of this population (Gayan et al., 1999). Sib-pair linkage analysis was performed using the Haseman-Elston algorithm implemented in the Genetic Analysis System (Young, 1995) with 20 new markers from 6p21.3 and with polymorphic sites in DAT1 and DRD4 in 310 sib pairs. As before, significant linkage was found with quantitative measures of component phenotypes of dyslexia and 6p21.3 markers when the families were selected for severity (e.g. $p=0.00053$ for the orthographic coding phenotype for families in which at least one child had a standard score <-2). Following DSM-IV criteria, ADHD was measured as the number of positive symptoms expressed, and is currently available on only 99 sib pairs. The ADHD phenotype showed linkage to markers in the 6p21.3 critical region ($p=0.0086$), but neither dyslexia nor ADHD showed linkage to the two dopamine-related loci. This would suggest that the ADHD symptoms in this population are not strongly influenced by these dopamine genes, and may be either secondary to reading disability or due to pleiotropic effects of the RD locus. Alternatively, there could be two tightly linked genes for RD and ADHD in the 6p21.3 region. Funded by NIH-NICHD HD27802.

A cluster of intronless genes in the mouse Prader-Willi syndrome candidate region. *J.H. Chai¹, D.P. Locke¹, T. Ohta¹, J.M. Greally², R.D. Nicholls¹.* 1) Genetics, Case Western Reserve Univ., Cleveland, OH; 2) Genetics, Yale Univ., New Haven, CT.

Prader-Willi syndrome (PWS) is a contiguous gene disorder associated with a 2 Mb domain of imprinted, paternally expressed genes in human chromosome 15q11-q13 and the orthologous mouse chromosome 7C. Among the putative protein-coding genes identified in the PWS region, two (*SNURF*, *SNRPN*) are encoded by a unique polycistronic mRNA that arose ~120-150 million years ago (mya) upon duplication of the ancestral *SNRPN'* gene, whereas the other four (*MKRN3/ZNF127*, *NDN*, *MAGEL2*, *C15ORF2*) are intronless. Intronless genes most commonly arise by retrotransposition from an intron-containing source gene. Such an event clearly generated *MKRN3* from *MKRN1* ~90 mya, while *C15ORF2* appears to be of recent (~45 mya) retroviral origin. Here, we have obtained additional evidence for recent evolutionary acquisition in this genomic region. BLAST searches using PWS-region gene sequences identified a series of partially sequenced BAC clones from mouse chromosome 7. Bioinformatic analysis of one selected BAC, 266F22, containing *Mkrm3*, *Magel2*, and *Ndn*, identified the intronless *Frat3* gene and the *Atp51P1* pseudogene in a 12 kb segment. Although the latter locus has not yet accumulated an inactivating mutation, there are no ESTs from this locus, it retains a polyA tail and is flanked by 15 bp direct repeats, hallmarks of retrotransposition. Restriction enzyme and PCR analyses of overlapping BACs 266F22 and 400O8 confirmed that *Frat3*, *Mkrm3*, *Magel2* and *Ndn* are tightly clustered in the mouse genome. By RT-PCR analysis of brain mRNA, *Frat3* is expressed solely from the paternal allele of wildtype and Angelman syndrome micw, with no expression from the maternal allele in PWS mice. *Frat3* may therefore play a role in phenotypic aspects of PWS mouse models. Current studies are ongoing to determine whether an ortholog of *Frat3* is present in human. In conclusion, the identification of five intronless genes in a small genomic interval suggests this region has an open chromatin structure in germ cells that is prone to integration and subsequent functional expression of foreign sequences.

Evidence for association/linkage disequilibrium between schizophrenia and DNA polymorphisms on chromosome 10p, a candidate region as suggested by linkage analysis. *S.G. Schwab¹, G.N. Eckstein¹, J. Hallmayer², S. Gabel¹, C.L. French³, M. Albus⁴, B. Lerer⁵, W. Maier¹, D.B. Wildenauer¹.* 1) Dept Psychiatry, University of Bonn, Bonn, Germany; 2) Graylands Hospital/University of Western Australia, Perth, Australia; 3) The Sanger Centre, Hinxton, UK; 4) Mental State Hospital, Haar, Germany; 5) Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

In a collaboration with clinical groups from Bonn and Haar, Germany, and Jerusalem, Israel, we have collected a sample of 71 families with schizophrenia each containing two or more affected siblings with parents for IBD analysis. Model free linkage analysis using GENEHUNTER revealed evidence for linkage on 10p (NPL=3.13; P=0.0015 centred around marker D10S211). Evidence for linkage in this region has been also shown by other independent studies in families with schizophrenia and by one study in families with bipolar disorders. Further support has been obtained in our family sample but also in an independent sample of 97 simplex families (one affected offspring plus parents) for D10S211 by transmission disequilibrium test (TDT). The exon/intron structure of a candidate gene close by, the gene for Human Phosphatidylinositol-4-kinase (PIP5K2A) has been determined by primer walking on BACs and confirmed by sequence data now available from databases. Nine of the 10 exons and the 3'UTR were screened by SSCP- and DHPLC-analysis. Two informative SNPs and one polymorphic microsatellite marker close to exon/intron boundaries have been identified and tested in the family sample by TDT. P-values of 0.038 (D10S211), 0.006 (SNP1), 0.01 (SNP2), 0.014 (GT-repeat) were detected. A distance of at least 200kb between D10S211 and the PIP5K2A gene was estimated from the BAC contig. Although the gene can be considered as an excellent candidate, the possibility remains that the novel polymorphisms indicate linkage disequilibrium with a gene in the neighborhood. (supported by Deutsche Forschungsgemeinschaft and German-Israeli Foundation).

Variation in the estrogen receptor genes (ESR1 and ESR2) and anorexia nervosa. *H. Eastwood*¹, *K.M.O Brown*¹, *D.A. Campbell*², *A.F. Markham*¹, *L.F. Pieri*³. 1) Molecular Medicine, University of Leeds, St James's University Hospital, Leeds, UK; 2) SmithKline Beecham Pharmaceuticals, Essex, UK; 3) Yorkshire Centre for Eating Disorders, Seacroft Hospital, Leeds, UK.

The aim of this study was to investigate the genetic contribution of variation in the ESR1 and ESR2 genes in the susceptibility to anorexia nervosa (AN). As women have higher estrogen levels than men variability in these receptors may account for the increased frequency of AN in women. Our AN cohort (170 female Caucasians) and a control population (150 female Caucasians) were genotyped for two polymorphisms in ESR2, G1082A and A1730G. A significant association was found between the 1082A genotype and AN ($\chi^2 = 11.46$, $p > 0.008$, d.f.= 1) such that individuals carrying the 1082A allele were approximately three times more likely to be present in the AN cohort than the control. There was no significant difference between the distribution of the alleles at A1730G between the AN and control cohorts. The dinucleotide repeat in the 5' untranslated region and two common RFLPs (*PvuII* and *XbaI*) in the intron 1/exon 1 region of ESR1 were also genotyped in the above cohorts. In the present study variation in ESR1 was not associated with AN.

The ESR2 A1082G polymorphism is silent and therefore unlikely to be of functional significance. This suggests a yet uncharacterised mutation in linkage disequilibrium with this polymorphism may form a genetic component of AN.

HDL levels in *ABCA1* heterozygotes are predicted by cholesterol efflux levels and are influenced by age. S.M.

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Plasma HDL cholesterol is inversely correlated with susceptibility to coronary artery disease(CAD). It has been postulated that this is primarily due to its role as a carrier in the transport of excess cholesterol from peripheral cells to sites of catabolism. However, a direct relationship between cholesterol efflux, HDL levels and CAD has not been demonstrated. We and others have recently identified mutations in the *ABCA1*(*ABCI*) gene as the cause of Tangier disease(TD), a disorder associated with a near absence of plasma HDL cholesterol due to defective efflux of cholesterol from cells. We have also reported that heterozygosity for *ABCA1* mutations is the cause of a dominantly inherited form of HDL deficiency(FHA) associated with reduced cholesterol efflux. We have now identified 13 *ABCA1* mutations in 11 families(5 TD,6 FHA) and have examined the phenotypic presentation of a cohort of 77 heterozygotes compared to unaffected family members. *ABCA1* heterozygotes have decreased HDL cholesterol(0.74 ± 0.24 vs. 1.31 ± 0.35 mmol/L, $p < 0.0001$) and increased triglycerides(1.66 ± 1.59 vs. 1.20 ± 1.03 mmol/L, $p = 0.03$), and display a greater than 3-fold increase in frequency of CAD(12.9 vs. 4.1%, $p = 0.03$) with earlier onset(48.9 ± 8.6 vs. 60.4 ± 12.8 yrs, $p = 0.08$) than unaffected family members. Levels of cholesterol efflux account for 82% of the variation in HDL levels in heterozygotes($p = 0.005$), and CAD is more frequent in heterozygotes with lower cholesterol efflux. Age modifies the phenotype in heterozygotes, with a much higher proportion of individuals aged 30-70 years having HDL <5th percentile for age and sex compared to persons <30 years of age(79 vs. 35%, $p = 0.004$). These data provide direct evidence that impairment of reverse cholesterol transport is associated with reduced plasma HDL cholesterol levels and increased CAD susceptibility, and provide further validation of *ABCA1* as a therapeutic target for prevention of CAD.

The characterization and genotyping of Single Nucleotide Polymorphisms in coding regions of brain-expressed Autism candidate genes. *L.R. Crawford, J. Bailey, B.L. Merriman, V. Kustanovich, S. Smalley, S. Nelson.* Department of Human Genetics, UCLA, Los Angeles, CA.

Autism is a neurological disorder with an occurrence of approximately 1/1000, characterized by severe impairments in language and social interaction. Autism is a complex genetic disorder involving an estimated 6 to 15 genes. Previous searches for these genes have relied on microsatellite markers and linkage analysis. These studies have identified large regions of linkage on chromosomes 4,7 and 16. In contrast to this approach, and to maximize our ability to locate autism-related genes, we are genotyping autistic subjects for a large set of functional SNP polymorphisms located in brain-expressed genes. Included in the gene set are four genes whose elevated protein levels have recently been shown to be highly correlated with autism: VIP (Vaso-Intestinal Peptide), BDNF (Brain Derived Neurotrophic Factor), NT 4/5 (Neurotrophin 4/5), and CRGP (Calcitonin Related Gene Peptide). Also included are genes involved in neurological development and the dopamine, serotonin, and adrenergic pathways as well as genes in areas shown to have linkage to Autism. SNPs in the candidate genes were located by combining data from dbSNP, the UCLA SNP database derived from UNIGENE sequence data, and from our own sequencing of genes in affected subjects. For example, functional SNP polymorphisms (causing amino-acid or promoter region variation) were located in BDNF, CGRP, VIPR2 (a VIP receptor), COMT, DRD1, DRD5, DRD3, ADRA1C and NCRAM and GRM8. We are initially screening subjects in 24 multiplex and 40 simplex Autism Families. We are SNP genotyping with two high throughput methods, a Fluorescent Polarization Assay developed in conjunction with LJI Biosystems, and a customized Affymetrix Tag Array. We will discuss the genes that were highlighted for further study in this original scan, as well as discuss the application of this methodology to identifying genes in other brain-related genetic disorders such as Attention Deficit Disorder and Schizophrenia.

The e4 allele for APOE doubles the risk of poor long-term prognosis in multiple sclerosis. *T. Masterman¹, J. Hillert¹, L. Lannfelt², E.H. Corder³.* 1) Neurology, Huddinge University Hospital, Huddinge, Sweden; 2) NEUROTEC, Geriatric Medicine, Novum, Huddinge University Hospital, Sweden; 3) Center for Demographic Studies, Duke University, Durham, NC.

The severity of multiple sclerosis (MS) was investigated in relation to apolipoprotein E (APOE) genotype, contrasting e34 (n=80) with e33 (n=75). Patients with a 'Benign' clinical course had EDSS scores of 0 to 4 after 10+ years disease duration, whereas patients with 'Severe' disability had scores between 6 and 9 regardless of disease duration. As expected, the 'Severe' group had an excess of e34 (44% vs 31%), but this tendency was not statistically significant (Fisher's exact test, one-sided; p=0.07). The odds of poor long-term, i.e. after 10+ years, prognosis were doubled for e34 carriers (p=0.048). Among severely disabled patients with long duration, mean EDSS was higher for e34, 7.9 vs 7.4 for e33, p=0.01 compared to 6.9 vs 6.7 for short duration. 'Benign' patients with e34 also had higher median EDSS, 3 vs 2.5 for e33, and had a broader spectrum of HLA alleles. In conclusion, the e4 allele for APOE may double the risk of poor long-term prognosis for MS. It is not a major risk factor for poor short-term prognosis. Subjects selected for benign and severe symptoms each demonstrated more disability with long duration when e4 was present, about half a point on the EDSS. APOE e4-related disability accumulated slowly over decades at a relatively constant rate.

Genotype/Phenotype Correlations Among the 5 Molecular Classes of Angelman Syndrome. *A.C. Lossie¹, M. Whitney¹, D. Amidon¹, A. Hutson², D. Theriaque², R.T. Zori¹, R.D. Nicholls³, C.A. Williams¹, D.J. Driscoll¹.* 1) RC Philips Unit, Peds Genetics and; 2) Dept of Statistics & GCRC, University of Florida, Gainesville; 3) Dept of Genetics, Case Western Reserve University, Cleveland, OH.

Genomic imprinting is a parent-of-origin epigenetic phenomenon that is intimately involved in Angelman syndrome (AS). All classical AS patients are characterized by mental retardation, lack of speech and specific movement and behavioral disorders. 5 mechanisms involving chromosome 15 are thought to account for all AS patients: Class I) 4 Mb interstitial deletions (68%); Class II) paternal UPD (7%); Class III) imprinting mutations (3%); Class IV) intragenic UBE3A mutations (11%) and Class V) no known defects (11%). Significant phenotypic differences are observed among our AS patients. Therefore, we sought to clarify the roles that other 15q11-13 genes may play in the different phenotypes associated with the 5 classes by examining our patients for 25 distinct parameters in the most extensive study of AS genotype/phenotype correlations (GPC) to date.

We restricted our GPC to 93 classical AS families examined by us and report that: 1) all 5 classes can be clinically distinguished, with differences most striking in pigmentation, growth parameters, achievement of developmental milestones, and severity, frequency and age at onset of seizures. 2) Class I patients are the most severely affected, while Classes II and III are the least. For example, Class I patients walked much later (mean = 4.6 yrs) than the other classes (means range from 2.5-2.9 yrs), and 91% of Class I, but only 14% of Class II and III patients had seizures. 3) We have found the highest UBE3A mutation frequency to date, as 50% of our patients (3/4 familial, 7/16 sporadic) with normal DNA methylation have mutations in the AS gene, and 4) Class V patients must inherit AS via a novel mechanism. We are currently searching for alternative causes of AS in these patients. In summary, our data clearly demonstrate that while UBE3A mutations are sufficient to cause AS, other 15q11-13 genes must also be involved in the development of seizures, language, growth, pigmentation and gross motor skills.

Genotype/Phenotype correlations in 60 spastic paraparesis families. *F. Martinez-Murillo¹, A. Choudhri¹, J. Devaney², M. Marino², E.P. Hoffman¹.* 1) Children's Research Institute, Center III, Children's National Medical Center. Washington, DC; 2) Transgenomic Inc., Gaithersburgh, MD.

HSP/FSP (hereditary spastic paraparesis/familial spastic paraparesis) is a very heterogeneous syndrome of the upper motor neurons. The "uncomplicated" form is characterized by hyperreflexia, spasticity, and weakness of the lower limbs. To date there are at least 15 different loci believed to be responsible for some type of HSP, either autosomal dominant (9), autosomal recessive (3), or X-linked (3). The initial gene causative of autosomal dominant HSP has recently been reported (Hazan et al., 1999). This gene, spastin, corresponds to the SPG4 locus in chromosome 2p21-p22, and is thought to be the most common autosomal dominant locus, based on limited genetic linkage studies done by us and others. To determine the frequency of spastin gene mutations in autosomal dominant HSP we tested all 17 exons of the spastin gene in our cohort of 60 familial spastic paraparesis families with putative autosomal dominant mode of inheritance. The analysis was done using WAVE®, denaturing high performance liquid chromatography (DHPLC), designed for mutation and polymorphism screening, and sizing applications. After completion of 60% of the spastin exons, we have found fifteen heteroduplexes indicative of possible mutations. Initial sequencing of these putative mutations, has shown a novel splicing mutation and a new missense mutation in two different patients. We present genotype/phenotype correlations in these mutation positive patients.

Family based association study of candidate genes regulating lipid and apolipoprotein levels on the island of Kosrae. Z. Han, S.C. Heath, D. Shmulewitz, S.B. Auerbach, M.L. Blundell, T. Lehner, J. Ott, M. Stoffel, J.M. Friedman, J.L. Breslow. Starr Center Human Genetics, Rockefeller University.

Candidate gene intragenic markers previously associated with abnormal lipid levels, including apo CIII(SstI), apoAII(MspI), cholesterol(chol) ester transfer protein(CETP/Taq1B), chol 7 α -hydroxylase(Cyp7/BsaI), hepatic lipase(DraI), microsomal triglycerides(tg) transfer protein(Hhpl), and apo E(HhaI), were determined in 1103 individuals of the Kosrae pedigree, and tested in a family based association study for contribution to lipid and apolipoprotein levels. Using likelihood estimation that account for the family structure to calculate genotypic means of lipid traits, significant results were (one is the common allele and 2 is the rare allele): Compared to apo CIII/SstI 11's, tg increased 9% in 12's ($p=0.0035$) and 25% in 22's ($p=0.0002$). Chol and apoB increased 6% ($p=0.0006$) and 7% ($p=0.0013$), respectively, in 22's. Compared to apo AII/MspI 11's, tg increased 54% ($p<0.0001$) and apoAI decreased 7% ($p=0.0097$) in 22's. Compared to CETP/Taq1B or cyp7/BsaI 11's, apoAI levels increased 6% ($p=0.0022$) and 12% ($p=0.0008$) in 22's, respectively. In the triallelic system for apo E(E4,E3,E2), compared to E3/3, chol and apoB levels increased 13% ($p=0.0455$) and 10% ($p=0.018$), respectively, and apoAI levels decreased 7% ($p=0.0406$) in E4/4's. Tg and apoB levels increased 8% ($p=0.003$) and 3% ($p=0.0450$), respectively, and apoAI levels decreased 5% ($p<0.0001$) in E4/3's. Chol and apoB levels decreased 15% ($p=0.0015$) and 16% ($p<0.0001$) in E3/2's. In Kosrae our results suggest a co-dominant apoCIII allele for tg and a recessive allele for chol and apoB, a recessive apoAII allele for increased tg and decreased apoAI, and recessive CETP and cyp7 alleles for apoAI. As expected the E4 allele is associated with increased tg, chol, apoB and decreased apoAI, whereas the E2 allele is associated with decreased chol and apoB levels. Compared to general population association studies, the family based association study described here is more likely to reveal true associations because it is less prone to false-positive association from population stratification and the subjects are living in a more homogenous environment.

Molecular assessment of 22q11.2 deletion in adults with schizophrenia or tetralogy of Fallot. A. Shi¹, L. Scutt², R. Weksberg³, E. Chow², M.A. Gatzoulis⁴, G.D. Webb⁴, A.S. Bassett², L.M. Brzustowicz^{1,5}. 1) Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ; 2) Schizophrenia Research Program, Queen Street Division, CAMH, Toronto, Canada; 3) Departments of Paediatrics and Genetics, The Hospital for Sick Children and University of Toronto, Canada; 4) Division of Cardiology, Toronto General Hospital, Canada; 5) Department of Psychiatry, UMDNJ, New Jersey Medical School, Newark.

Patients with 22q Deletion Syndrome (22qDS) have deletions of 22q11.2 and variable phenotypic characteristics including mild dysmorphic features, hypernasal speech and learning difficulties. About 25% of adults with 22qDS have schizophrenia. We studied 31 adults with schizophrenia (SZ) with clinical features suggestive of 22qDS and 61 adult subjects with tetralogy of Fallot (TOF) but no history of schizophrenia. Both sets of subjects were of similar northern European Caucasian ancestry. Standard FISH testing detected deletions in 19 SZ subjects. Among 12 TOF subjects with additional clinical symptoms suggestive of 22qDS, only 7 had deletions detected by FISH. Loss-of-heterozygosity analysis was conducted with 12 microsatellite markers spanning the 22qDS deletion region. This identified one additional TOF subject with a deletion. Among SZ subjects with FISH-confirmed deletions, a common region of homozygosity/hemizyosity extended from marker D22S941 to D22S1709, with individual regions extending as proximal as D22S420 and as distal as D22S257. A common region of homozygosity/hemizyosity in TOF subjects with FISH-confirmed deletions extended from marker D22S941 to D22S1648, much smaller than the region in SZ subjects, although individual deletions extended as far as in the SZ subjects. Comparing SZ and TOF subjects with a FISH-confirmed deletion or a high probability of deletion based on multiple adjacent homozygous markers (n=26 in each diagnostic group), significantly more homozygosity/hemizyosity was seen in the SZ group for the markers D1S264, D1S311, and D1S1709. We are conducting additional FISH analyses and genotyping additional subjects and markers from this region to better define the differences in deletion region between these two groups.

POF2 GENE MAY BE RESPONSIBLE FOR THE OVARIAN PHENOTYPE OF TURNER SYNDROME. S.

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A new gene, POF2, was identified by bioinformatic tools in the critical region for POF (Premature Ovarian Failure, OMIM 311360) in Xq21. The POF2 gene encodes a 589 aa putative protein with a large coiled-coil domain and no other homologies with known proteins. The new gene was found interrupted in the third intron by the breakpoint of a balanced X/1 translocation of a POF affected woman. Interestingly, RT-PCR experiments revealed the presence of a normal transcript in the patient which was not expected as the normal X chromosome is inactivated in balanced translocations. Investigation of the inactivation pattern of this new gene by RT-PCR amplification on different hybrid cell lines containing only the active or the inactive X chromosomes indicates that the POF2 gene is not subjected to X-inactivation. This evidence makes the POF2 gene a good candidate not only for the POF disease but also for the ovarian dysgenesis peculiar of the Turner Syndrome (TS). TS is associated with X chromosome monosomies and is a general belief that haploinsufficiency of specific genes normally required in double dosage is the cause of the pathology. Moreover the mutation analysis of the POF2 gene in a collection of 170 POF females with normal karyotype, revealed the presence of two missense mutations in three different patients with early onset of the disease. Taken together these results indicate that POF2 gene is a strong candidate for the normal development and/or function of the ovary.

Lack of association of MICA transmembrane region polymorphism and Behet's disease in Tunisian population.

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Behçet's disease (BD) is a systemic disorder mainly characterized by recurrent oral ulcers, genital ulcers, uveitis and skin lesions. Although the etiopathogeny of BD is still unknown, it may involve environmental as well as genetic factors. HLA-B51 has been reported as a genetic marker associated with BD in several ethnic groups. However, it is not clear whether HLA-B51 itself or a closely linked gene is responsible for susceptibility to BD. Recently, MICA (MHC class I chain-related gene A) has been proposed as a possible candidate gene for BD susceptibility given its location and putative function. Analysis of a triplet repeat polymorphism in the transmembrane region of MICA in Japanese patients revealed that A6 MICA-TM was in strong linkage disequilibrium with HLA-B51 allele and may be more closely associated with BD than HLA-B51. However, analysis of other ethnic groups has not always confirmed this association. In the present study, we have investigated the distribution of MICA-TM alleles and HLA-B51 in 41 Tunisian BD fulfilling the International Criteria and 43 ethnically matched controls. As previously reported, HLA-B51 frequency was increased among patients with BD (48.7% vs 25.5% in healthy controls, $p < 0.05$). Allele frequency of A6 MICA-TM allele was slightly but not significantly increased in the patient's group as compared to the control group (85.3% vs 74.4%, $p > 0.05$). No significant differences were found between patients and controls for A4, A5, A5.1 and A9 MICA-TM allele frequencies. 95% and 100% of HLA-B51 positive patients and controls, respectively, carry A6 allele ($p > 0.05$). In HLA-B51 negative individuals, A6 allele was present respectively in 76.1% and 65.6% of patients and controls ($p > 0.05$). These data suggest that HLA-B51 is more closely associated to BD than A6 MICA-TM allele in Tunisian population. HLA-B51 could be directly implicated in the susceptibility to BD. However, involvement of other genes close to HLA-B locus can not be ruled out.

Analysis of the involvement of the 2 bp deletion polymorphism of the alpha7 nicotinic acetylcholine receptor gene in P50 sensory gating deficit associated to schizophrenia. *F. Bonnet-Brilhault^{1,2}, G. Raux², S. Louchart¹, E. Houy^{1,2}, M. Petit^{1,2}, F. Thibaut^{1,2}, D. Campion^{1,2}, T. Frébourg².* 1) Service Hospitalo-Universitaire de Psychiatrie, CHU de Rouen et CHSR du Rouvray; 2) INSERM EMI 9906, Faculté de Médecine et de Pharmacie IFRMP, 76183 Rouen, France.

The P50 auditory-evoked potential gating deficit is an endophenotype associated with the expression of schizophrenia. Biochemical and genetic studies have suggested that the alpha7 nicotinic acetylcholine receptor belongs to an inhibitory neuronal pathway involved in this phenotype. A 2-bp deletion polymorphism has previously been reported within exon 6 of the alpha7 nicotinic acetylcholine receptor gene, *CHRNA7*, located on chromosome 15. This deletion, resulting into a premature stop codon, potentially generates a non functional alpha 7 subunit and might be related to an abnormal P50 inhibition. However, the analysis of this polymorphism is not straightforward since the *CHRNA7* gene is partially duplicated. To distinguish all possible genotypes, we developed a simple PCR-based assay using dye-labeled primers. We recorded P50 auditory-evoked potential and analyzed this polymorphism in 175 schizophrenic cases (DSM-III-R criteria) and 172 controls. No significant difference in the distributions of genotype frequencies was observed between patients and controls ($\chi^2=0.912$, $df=2$, NS). However, preliminary results cannot exclude a difference in the genotype distributions between subjects with an abnormal P50 ratio and subjects with a normal P50 ratio.

Association of late-onset familial Alzheimer disease (AD) and single nucleotide polymorphisms (SNPs) in the LRP1 gene. *J.M. Grubber¹, W.K. Scott¹, E.H. Lai², A.M. Saunders¹, K.A. Desombre¹, P.M. Conneally³, G.W. Small⁴, A.D. Roses², J.R. Gilbert¹, J.M. Vance¹, J.L. Haines⁵, M.A. Pericak-Vance¹.* 1) Duke University Medical Center, Durham, NC; 2) GlaxoWellcome Research & Development, Research Triangle Park, NC; 3) Indiana University School of Medicine, Indianapolis, IN; 4) University of California, Los Angeles, CA; 5) Vanderbilt University Medical Center, Nashville, TN.

The low density lipoprotein receptor-related protein (LRP1) gene is an attractive candidate gene for late-onset familial AD because it functions as a receptor for apolipoprotein E and is located in a region on chromosome 12 recently linked to AD. Therefore, several investigators have examined single polymorphisms in LRP1 for linkage and association to AD, producing conflicting results. The recent interest in using haplotypes of SNPs for candidate gene analysis prompted a re-examination of LRP1 in an expanded data set. We examined 266 discordant sibships (DSPs; ≥ 1 affected with late-onset (≥ 60) AD and ≥ 1 unaffected) ascertained by Duke University Medical Center, UCLA, Indiana University, and the NIMH AD Genetics Initiative. Four SNPs, located in introns 38 and 78 and exons 44 and 54, were genotyped using oligonucleotide ligation assay (OLA). Two programs, the Pedigree Disequilibrium Test (PDT), a single-locus test of linkage disequilibrium in general pedigrees, and TRANSMIT, a multilocus test of linkage disequilibrium in nuclear pedigrees, were used to test for association. Moderate evidence for association was detected at the exon 54 SNP using both the PDT ($p=0.06$) and TRANSMIT ($P=0.03$). Tests of the other 3 SNPs were not statistically significant using either test. However, analysis of all 4 SNPs as a haplotype using TRANSMIT produced a significant global chi-square test of association ($p<0.001$). These results demonstrate that evidence for association of AD and LRP1 increases when considering haplotypes of LRP1 intragenic SNPs. These data are consistent with previous studies indicating that one or more AD susceptibility genes are located in the pericentromeric region of chromosome 12.

DOVAM-S: Evidence for generic scanning conditions and application to the steroid receptor gene family. J.

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We recently developed [Detection of Virtually All Mutations]-SSCP (DOVAM-S), a modification of SSCP in which 23 amplicons from genomic DNA are analyzed in a single lane with five different electrophoresis conditions. To determine whether the scanning conditions were generic, blinded analyses were performed in two genes, factor VIII and ATM. All 180 mutations and polymorphisms were detected. The redundancy of electrophoresis conditions is sufficient to detect virtually all mutations. Parallel analysis of 48 samples with multiple exons per lane allows 500 kb to be scanned with five gels. DOVAM-S was utilized to scan the regions of likely functional significance of the steroid receptor gene family in patients with psychiatric diseases. Steroid and thyroid hormone administration can alter behavior dramatically, which suggests that genetic variants may be predisposed to psychiatric disease. To investigate this possibility, the glucocorticoid receptor (GR) gene from 100 patients with schizophrenia and 40 patients with puerperal psychosis, and the estrogen receptor α (ER α) gene and the thyroid receptor α (TR α) gene from 240 subjects (including 113 schizophrenic, 28 bipolar disorder, 24 puerperal psychosis, 25 autistic, 25 ADHD, and 25 alcoholic subjects) were scanned by DOVAM-S. A total of 3.3 megabases were scanned. Five missense and four silent mutations were identified in the GR gene; four missense and seven silent mutations were identified in the ER α gene; only four silent mutations were present in the TR α gene. Two of the missense mutations in ER α are conserved in the six available mammalian and bird species and a third sequence variant is conserved in mammals, birds, and *Xenopus*, hinting that these sequence changes will be some of functional significance. These changes were found in one patient each with bipolar disorder, puerperal psychosis and alcoholism, respectively.

Role of Reduced Folate Receptor Polymorphisms in NTD Risk. *M.W. Baker¹, G.M. Shaw², E.J. Lammer³, R.C. Barber¹, W. Xiong¹, F.J. Aleman¹, R.H. Finnell¹.* 1) Center for Human Molecular Genetics, University of Nebraska Medical Center, Omaha, NE; 2) California Birth Defects Monitoring Program, Emeryville, CA; 3) Children's Hospital Oakland, Oakland, CA.

Neural tube defects (NTDs) are multifactorial in their etiology, having both genetic and environmental factors contributing to their development. Recent evidence demonstrates that periconceptional supplementation of the maternal diet with a multivitamin containing folic acid significantly reduces the occurrence and recurrence risk for having a pregnancy complicated by NTDs. Unfortunately, the mechanism underlying the beneficial effects of folic acid remain unknown. Current research is focused on the discovery of genetic abnormalities in folate transport genes, which may explain the population burden of these birth defects. One of the candidate genes is reduced folate carrier (RFC1) gene, which encodes a folate transporter. RFC1 mediates transport of the folic acid family of compounds, which are critical for de novo synthesis of purines and thymidine in mammalian cells. This project was designed to screen the whole RFC1 gene coding sequence in order to detect mutations in the patients with NTDs by direct sequencing on an ABI Prism 3700 DNA Analyzer. A G-to-A transition in exon 2 of RFC1 gene was identified in 8 of 19 NTDs patient's DNA samples that were collected from Texas Hispanic population. This single nucleotide substitution results in an amino acid change from arginine to histidine at position 27, which is adjacent to first one of 12 predicted transmembrane domains. The importance of this observation was examined in a population based, case-controlled study of NTDs conducted in California involving over 100 NTD cases. Analysis of this data continues to support the importance of periconceptional folic acid supplementation as a preventative measure to reduce the population burden of NTDs. Supported in part by NIH grant ES09106 and Centers of Excellence for Surveillance Research Service and Evaluation aimed at Birth Defects, Cooperative Agreement U59/CCU913241.

Mutation screen of the human $\alpha 7$ neuronal nicotinic receptor gene promoter in normal and schizophrenic individuals. *J.M. Gault¹, J. Logel^{1,2}, J. Hopkins², R. Vianzon², M. Short², M. Robinson¹, C. Drebing¹, R. Berger¹, K. Walton¹, B. Sullivan¹, R. Freedman^{1,2}, S. Leonard^{1,2}.* 1) Dept Psychiatry, Univ Colorado Health Sci Ctr, Denver, CO; 2) VA Medical Center, Denver, CO.

Biological and genetic evidence supports the hypothesis that the $\alpha 7$ receptor (CHRNA7) is involved in a sensory processing deficit found in many schizophrenics. Single strand conformation polymorphism (SSCP) and sequence analyses of the proximal human $\alpha 7$ promoter region, 248 bp upstream of the ATG translation start site, were used to identify variants in 292 samples from schizophrenics from 166 families and 164 samples from controls. The samples are approximately 60% Caucasian, 30% African American, 3% Hispanic and the remaining 7% are Pacific Islander, Asian, mixed or unknown ethnicity. To date, twelve nucleotide variants have been identified ranging in frequency from 1-21% in unrelated individuals. Five variants —191G®A, —180G®C, —178delG, —143G®A, and —86C®T were found more frequently in samples from schizophrenics than controls. One sample from a schizophrenic had both the —191 and the —178 variant. Four variants —194G®C, —190insG, —166C®T, —93C®G were found more frequently in samples from controls than schizophrenics and three variants —241A®G, —92G®A and —46G®T were found with similar frequencies in samples from both controls and schizophrenics. Five polymorphisms at positions —194, —191, —180, —178delG, —143 are located in consensus Egr-1 and Sp1 binding sites. Variant sites at —180, —178, and —143 are in conserved Egr-1 and Sp1 transcription factor binding sites that are functional in the bovine promoter. The others including —241, —190insG, —166, —93, —92, —86, and —46 variants are located in or near consensus sequences for transcription factor binding sites. Functional analysis of polymorphisms at —191A, —178delG, and —86T using a luciferase reporter gene transcription assay, shows a statistically significant drop in the transcription activity in transfected SHSY-5Y neuroblastoma cells. The promoter variants may reduce transcriptional activity for the in vivo $\alpha 7$ subunit and potentially contribute to the decrease in $\alpha 7$ receptors, seen in postmortem brain of schizophrenics.

Transgenic models of imprinting, protein production, and phenotypic rescue in Prader-Willi syndrome. *T. Ohta, T.A. Gray, C. Limwongse, B.T. Lamb, R.D. Nicholls.* Genetics, Case Western Reserve Univ, Cleveland, OH.

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are neurobehavioral disorders resulting from the loss of function of imprinted genes in human chromosome 15q11-q13. We have previously generated a PWS/AS mouse model, with a 4 Mb deletion of the PWS/AS-homologous region in mouse chromosome 7C. Mice with a paternal deletion have a failure-to-thrive similar to PWS, with early postnatal lethality. PWS is a contiguous gene disorder, with an unknown number and identity of etiological genes. One key genetic locus in PWS encodes the bicistronic *SNURF-SNRPN* transcript. *SNURF* is the only protein-coding sequence in the imprinting center (IC) that controls imprinting in PWS and AS, whereas *SNRPN* encodes a spliceosomal protein, SmN. Intriguingly, the absence of SmN in PWS brain is compensated by up-regulation of SmB/B' to maintain spliceosome integrity and ameliorate the PWS phenotype. Therefore, we generated cDNA and genomic transgenes for the complex IC/*SNURF-SNRPN* locus to examine its role in imprinting and PWS.

A BAC transgene (Tg) included 50 kb of 5' IC sequence and *Snurf-Snrpn*, with a unique 3' tag. This Tg was imprinted, as shown by paternal mRNA expression and maternal DNA methylation patterns. Both SNURF and SmN proteins were translated *in vivo* from the BAC Tg, after paternal inheritance, but do not rescue the PWS failure-to-thrive. Interestingly, a *SNURF* cDNA Tg expressing low levels of mRNA was post-transcriptionally silenced. A *SNURF-SNRPN* YAC Tg was hypomethylated at the *SNURF-SNRPN* CpG-island and non-imprinted, likely as a consequence of a small deletion identified in the 5' IC (including an exon of an alternative *SNRPN* transcript). Nevertheless, at present we cannot rule out lack of recognition of the human Tg by mouse regulatory proteins. Preliminary data suggests that this Tg may ameliorate the viability of PWS mice, and hence may contain a gene involved in the postnatal phenotype. In conclusion, these Tg's provide insight into the imprinting mechanisms and genes involved in PWS.

Monoaminergic genes and Schizophrenia among Indians: A family based association study. *B.K. Thelma¹, P. Semwal¹, S. Prasad¹, T. Bhatia¹, S.N. Deshpande², J. Wood³, V.L. Nimgaonkar³.* 1) Department of Genetics, Univ. of Delhi South Campus, New Delhi, India; 2) Dept.Of Psychiatry, Dr.R.M.L. Hospital, New Delhi, India; 3) Dept.of Psychiatry and Human Genetics, Univ.of Piitsburgh School of Medcine and Graduate School of Public Health, Pittsburgh.

Schizophrenia is a common disorder with a complex etiology . Despite segregation analysis in several independent samples, the mode of inheritance is unclear. In addition, linkage studies have been hampered by inadequate numbers of large multiply affected families or affected sibpairs. If this disorder is multifactorial /polygenic, the genetic susceptibility may represent the cumulative effect of several genes of relatively small effect. Association studies may therefore be useful,if they are sufficiently large. Family based association studies using the Transmission disequilibrium test(TDT) have emerged as the favoured design instead of the conventional case-control approach, which may be plagued by spurious associations due to population stratification.

This study reports on associations tested among North Indian patients with Schizophrenia and their parents (DSM IV criteria; n=179 families; n=568 participants).To our knowledge, only one family based association study has been conducted among Indians. Investigation of diverse ethnic groups may enable detection of novel associations hiterto undetected among caucasians, the ethnic group most intensively investigated for schizophrenia. Several genes involved in the dopaminergic and serotonergic neurotransmission pathways have been implicated in the etiology of Schizophrenia among caucasians. Polymorphism at four such putative loci namely Serotonin2A receptor (HT2A), Trytophan hydroxylase (TPH), Catechol-o-methyl transferase(COMT) and Dopamine transporter(DAT) were studied. Polymorphisms in both the gene and promoter at HT2A and TPH were investigated, thus enabling haplotype based analysis. Informative transmissions were tested using the TDT . No significant associations were detected with any of the markers.

Generalised epilepsy with febrile seizures plus: Mutation of the sodium channel subunit SCN1B and evidence for a founder effect. *R.H. Wallace¹, I.E. Scheffer^{2,3}, S. Barnett¹, S.F. Berkovic^{2,3}, G.R. Sutherland^{1,4,5}*. 1) Cytogenetics & Molecular Genetics, Women's & Children's Hospital, North Adelaide, SA, Australia; 2) Department of Medicine (Neurology), University of Melbourne, Austin & Repatriation Medical Centre, Heidelberg, VIC, Australia; 3) Royal Children's Hospital, VIC, Australia; 4) Department of Genetics, University of Adelaide, Adelaide, SA, Australia; 5) Department of Paediatrics, University of Adelaide, Adelaide, SA, Australia.

Generalised epilepsy with febrile seizures plus (GEFS+) is a common childhood genetic epilepsy syndrome with heterogeneous phenotypes ranging from febrile seizures to generalised epilepsies of variable severity. We recently described linkage to chromosome 19q13.1 and identification of a mutation (C387G) in the voltage-gated sodium channel $\beta 1$ subunit gene (*SCN1B*) in a large GEFS+ family. The mutation changes a conserved cysteine residue (C121W) disrupting a putative disulfide bridge maintaining an extracellular immunoglobulin-like fold. We now report a second GEFS+ family with a C121W mutation within the sodium channel $\beta 1$ subunit, SCN1B. The family had 18 affected individuals, nine with febrile seizures and nine with febrile seizures plus. Twelve affected individuals, out of 15 tested, carried the mutation, as did three obligate carriers and one unaffected family member. Haplotype analysis suggested a founder mutation although genealogical records failed to reveal any recent relationship between this and the original family. Ascertainment of the second family confirms the association between the C121W mutation of SCN1B and GEFS+.

Characterization of the necdin gene family and its role in Prader-Willi syndrome. R. Wevrick¹, S. Lee¹, S. Kozlov², J.M. Bischof¹, S.L. Kuny¹, T.K. Chibuk¹, L. Hernandez², C.L. Stewart². 1) Dept Medical Genetics, Univ Alberta, Edmonton, AB, Canada; 2) Lab. of Cancer and Devel. Biol., NCI-FCRDC, Frederick, MD.

Two members of the necdin gene family, *NDN* (necdin) and *MAGEL2* (*NDNLI*) are imprinted genes located 41 kb apart in the region deleted in the neurodevelopmental genetic disorder Prader-Willi syndrome (PWS). Affected individuals exhibit neonatal hypotonia, developmental delay and childhood-onset obesity. Necdin, a protein implicated in the terminal differentiation of neurons, is the only PWS candidate gene to reduce viability when disrupted in a mouse model. *MAGEL2* expression was detected predominantly in brain, the primary tissue affected in PWS, and in several fetal tissues, by northern blot analysis. Distribution of the orthologous mouse transcript (*Magel2*) partially overlaps that of *Ndn*, with strong expression in the central nervous system in mid-gestation mouse embryos by whole mount RNA *in situ* hybridization. However, *Magel2* expression diminishes by late gestation, whereas moderate expression of necdin remains through late gestation and in the adult. Necdin localization was primarily nuclear but *MAGEL2* was cytoplasmic in transient transfections of HEK cells with GFP reporter constructs. Ongoing studies of necdin and *Magel2* include immunolocalization and identification of interacting factors. Both genes are candidates for the neurological phenotype, based on the role of necdin in murine neuronal differentiation and expression of both genes in the developing mouse brain. We hypothesize that although loss of necdin expression may be important in the neonatal presentation of PWS, simultaneous loss of *MAGEL2* may also be critical to abnormalities in brain development and dysmorphic features in PWS. We have also identified two additional members of the necdin gene family by database searches. *NDNL2* is located on chromosome 15 but outside the PWS deletion region. *NDNL2*, its murine homologue *Ndnl2* and another mouse gene *Ndnl3* each share significant amino acid identity with *NDN* and *MAGEL2* in the carboxy terminus. Investigation of the expression patterns of other members of the necdin gene family will clarify how loss of *NDN* and *MAGEL2* activity may contribute to PWS.

Significant association between CD19 polymorphism and systemic lupus erythematosus. *K. Tokunaga¹, K. Kuroki¹, N. Tsuchiya¹, A. Yamaguchi¹, B.P. Tsao², J.M. Grossman², T. Fukazawa³, H. Hashimoto³.* 1) Dept Human Genetics, Univ Tokyo/Grad Sch Medicine, Tokyo, Japan; 2) UCLA, Los Angeles, CA, USA; 3) Juntendo Univ., Tokyo, Japan.

CD19, a 95kDa B lymphocyte surface antigen, is known to regulate the signaling for B lymphocyte development, activation and proliferation. CD19-deficient mice have a significant decrease in the B cell proliferation and in the serum immunoglobulin levels. In contrast, transgenic mice that overexpress CD19 were demonstrated to be hyperresponsive to signals from B cell receptor and to show autoantibody production. Furthermore, human CD19 is located at chromosome 16p11.2, and this position has been suggested to be one of the candidate loci for rheumatoid arthritis (RA). In the present study, we considered CD19 as a strong candidate for a susceptibility gene to RA and systemic lupus erythematosus (SLE), and performed the variation screening of the entire CD19 coding region by a PCR-SSCP method, using the genomic DNA from healthy Japanese individuals and patients with RA and SLE. Through the variation screening, three single nucleotide polymorphisms (SNPs), nt406C/T (L136L), nt520G/C (V174L) and nt705G/T (P235P), were identified within the coding region, and two rare variations were found within introns flanking the exon-intron junctions. Among these variations, nt705T (P235P) was observed with a significantly lower frequency in the patients with SLE (25/90, 27.8%) compared with that in healthy individuals (109/247, 44.1%) ($P=0.0085$). No association was observed for RA. In order to test whether the negative association of nt705T with SLE is present in other populations, 107 Caucasian SLE family samples from southern California were also examined using transmission disequilibrium test. A tendency of preferential nontransmission of nt705T allele was observed, although statistically not significant. These results for the first time suggested that nt705T may confer protection against the development of SLE Japanese. Further variation screening in the promoter and 3'-untranslated regions to find other SNPs which might be in linkage disequilibrium with nt705T is underway.

Association of asthma and related phenotypes with ASTH1-I/J genes on chromosome 11p in Caucasians. *G.P Sreekumar¹, C.G Binnie¹, E.G Carden¹, S. Sharma¹, C.S Sprankle¹, P.A Sherman¹, Z.Y Xue¹, M.E Fling¹, M. Reisman¹, M. Blumenthal², J. Vestbo³, J. Sundy⁴, M.A Pericak-Vance⁴, S. Brewster¹, M.G Ehm¹, D.K Burns¹, M.J Wagner¹.* 1) Glaxo Wellcome, Inc, Research Triangle Park, NC; 2) Department of Medicine, University of Minnesota Medical School, Minneapolis, MN; 3) Department of respiratory Medicine, University of Copenhagen, Denmark; 4) Duke University Medical Center, Durham, N.C.

ASTH1 I and ASTH1 J genes are reported to be associated with asthma (Patent application WO 99/37809 filed by AXYS pharmaceuticals) in Tristan de Cunha and Toronto populations. These genes mapped on chromosome 11p12 are expressed at high levels in trachea and prostate and low levels in lungs and kidney, and belong to the ETS family of transcription factors. We genotyped 13 SNPs and one microsatellite marker in 89 Caucasian nuclear families with at least one offspring affected with asthma (physician's diagnosis) from Minnesota, 197 nuclear families from Denmark, and 200 cases and 100 controls from a US Caucasian sample from North Carolina. We investigated the evidence for association with asthma using several phenotypes: diagnosis by a physician, strict asthma (2 or more symptoms and a positive methacholine challenge test or broncho-dilator test) and bronchial hyper-responsiveness (positive methacholine reaction at or below 10mg/ml of methacholine). TDT and case control analysis detected significant association of 7 markers with asthma and related phenotypes in the Minnesota collection. Association was detected with 2 markers each in the Denmark and US Caucasian case control collections. Haplotype analysis also indicated significant association of several haplotypes in this region. Linkage disequilibrium (LD) analysis between the markers suggests that the LD extends about 100kb in the ASTH I and ASTH J region. The results of our analysis replicate the association of asthma with ASTH I/J genes or the genomic region containing these genes.

Development of Asthma-Immuno Chip for gene expression studies of asthma and other immune mediated diseases. A. Polvi¹, A. West², R. Kinos², P. Huhtinen², Y. Qin², R. Lund², T. Laitinen¹, J. Kere¹, R. Lahesmaa². 1) Haartman Institute, Department of Medical Genetics, University of Helsinki, Finland; 2) Turku Centre for Biotechnology, Turku, Finland.

It has been estimated that approximately 200 million people worldwide suffer from asthma. In developed countries 5-7% of the population have asthma, and the frequency of asthma and allergy are increasing, reaching 10-15% in children. In Finland, there are ~160,000 asthma patients who receive a specific compensation for their therapy. Since the pathological mechanism leading to asthma is mainly unknown, current therapies are primarily targeted to relieve the end-symptoms. There is a significant medical need for improved diagnostics and targeted disease-modifying therapy. We developed a tool for the expression analysis of genes, signaling pathways and disease-related molecular networks critical for the development of asthma and other atopic disorders. We chose 442 genes that are potentially involved in asthma pathogenesis and 1458 genes involved in cellular signaling networks implicated in allergic and immune mediated diseases. Of the chosen genes, 1562 were picked up from the Research Genetics Sequence Verified Human cDNA Clone set. In addition, 189 IMAGE clones, not included in the set, were sequence verified. Also, 149 genes that were not commercially available were cloned. Altogether 1900 clones were collected and printed into poly-lysine coated glass slides. The created Asthma-Immuno Chip contains cDNAs encoding e.g. cytokines, chemokines, receptors for cytokines and chemokines, adhesion molecules, signaling proteins, transcription factors, apoptosis-related proteins, cell structural components and enzymes, and will be used for gene expression analysis of asthma and other immune mediated diseases.

Identification of pharmacogenetically relevant cDNAs using mRNA-differential display and genetic strains of mice with special reference to alcohol response. *J.A. Treadwell, K.R. Uddin, M.L. Harrison, S.M. Singh.* Zoology, University of Western Ontario, London, Ontario, Canada.

Strains of mice exhibiting pharmacogenetic differences represent a valuable resource for analysis and identification of specific genes using a variety of approaches including linkage-based QTL mapping. RNA-based molecular methods now permit the identification of differentially expressed genes in a suitable system, and their subsequent assignment to an appropriate phenotype. In this ongoing study we have concentrated on four strains of mice (C57BL/6J, A/J, BALB/c and DBA/2J) that are known to differ for a variety of phenotypes including preference for and response to alcohol. We have collected brain RNA from ethanol-treated male (n=4) and female (n=4) mice from these strains and their matched controls. This RNA is being used in differential displays with a large number of primer combinations. To date we have cloned and sequenced 38 differentially expressed partial cDNAs: 25 of these represent strain differences and 13 are responsive to acute ethanol treatment. Approximately 50% of these sequences find matches in databases (genes of interest include dystrobrevin, neurochondrin, and several EST and genomic sequences of unknown function); the rest potentially represent novel neurally-expressed genes. We are using 5'RACE to obtain the complete sequences, and are evaluating the co-segregation of candidate cDNA expression with alcohol preference (voluntary alcohol consumption, VAC) in appropriate F2 and RI lines. The human homologs of such murine cDNAs will represent logical candidate genes involved in alcohol preference and the risk for the development of alcoholism. Similar approaches could also help identify genes important in a number of other addictive phenotypes.

Familial correlations and heritability of serum IL-4 level. *M.B. Freidin¹, I.M. Kulmanakova², L.M. Ogorodova², V.P. Puzyrev^{1, 3}*. 1) Population Genetics Lab, Research Institute for Medical Genetics, Tomsk, Russia; 2) Pediatric Department, Siberian State Medical University, Tomsk, Russia; 3) Medical Genetics Department, Siberian State Medical University, Tomsk, Russia.

Familial correlation analysis and heritability estimation were carried out for serum interleukin-4 (IL-4) level, the key cytokine in allergic inflammation. The study was performed on the familial sample of Russian patients with atopic bronchial asthma and their relatives (35 families, n=120). IL-4 level in blood serum was measured by ELISA. After age, sex, and affection status adjustment the significant correlation on IL-4 level was obtained in 33 “mother-child” pairs ($r=0,36\pm0,16$; $p=0,03$), that along with the absence of significant correlation in 22 “father-child” pairs could mean “a maternal effect” in the studied trait variability. Correlation coefficient in 55 “parent-child” pairs was significant and equal $0,26\pm0,13$ ($p=0,04$). So, heritability grade for IL-4 level came to 52,2 %. It suggests that only a half of individual dispersion of the trait may be attributed of genetic variability and a significant part of residual variance is explained by environmental causes. It consists with the nature of the cytokine, controlling by genes, but stimulating by antigenic signal. This findings may propose additional insight in our understanding of gene-environment interactions in determination of traits underlying asthma and other complex diseases.

Three SNPs in the promoter region of interleukin-4 receptor gene are associated with atopic dermatitis in Japan.

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Interleukin-4 receptor alpha chain (*IL4R*) has been focused as a candidate gene for atopic diseases. Coding polymorphisms of the gene have been reported to be associated with atopic diseases with altered function. We now identified three promoter polymorphisms of *IL4R* gene: A-327C, C-326A, and A-186G (numbered from transcription initiation site). These three polymorphisms were in linkage disequilibrium and divided into two haplotypes: alpha (...ac...a...) and beta (...ca...g...). The table below shows the genotypes of the three patients groups: atopic dermatitis, and normal group in Japanese population. We have found that these polymorphisms are also present in Caucasian people.

	alpha / alpha	alpha / beta	beta / beta
atopic dermatitis	71	26	3
normal	34	29	2

The alpha / alpha genotype is weakly associated with atopic dermatitis ($p=0.0202$). Luciferase assay revealed that alpha haplotype has 1.5 times stronger promoter activity than beta haplotype. Furthermore, real-time quantitative RT-PCR analysis showed higher expression level of whole blood samples in atopic dermatitis group. Therefore, it is logical to think that this higher expression level of *IL4R* in atopic dermatitis is due in part to these functionally significant SNPs in promoter region of the gene.

Association of the tumor necrosis factor-alpha (TNF-a) and its receptor 2 (TNFR2) genes with human narcolepsy. *H. Hohjoh¹, N. Terada¹, M. Kawashima¹, Y. Honda², K. Tokunaga¹.* 1) Human Gen/Grad School Medicine, Univ Tokyo, Tokyo, Japan; 2) Seiwa Hospital, Neuropsychiatric Research Institute, Tokyo, Japan.

Narcolepsy is a sleep disorder characterized by recurrent daytime sleep episodes and cataplexy, and also a multifactorial disorder in which genetic and environmental factors appear to be involved. A genetic factor strongly associated with the disorder has been found in the HLA class II region: the HLA-DRB1*1501-DQB1*0602 haplotype predisposes to narcolepsy. We have been searching for other genetic factors associated with narcolepsy, and our previous study, where the single nucleotide polymorphisms (SNPs) in the tumor necrosis factor-alpha (TNF-a) gene were investigated in narcoleptic patients and healthy controls, suggested that TNF-a could be a new susceptibility gene for human narcolepsy. In this study, we investigated a relationship between the tumor necrosis factor receptor 2 (TNFR2) gene and human narcolepsy. The gene has the SNP that participates in an amino acid substitution at position 196, i.e., generates two alleles having methionine (196M) and arginine (196R). Based on the SNP, we typed the TNFR2 gene in 149 narcoleptic patients and 204 healthy controls. As a result, the frequency of the TNFR2-196R allele was elevated in the patients as compared with that in the controls. The difference in the TNFR2-196R frequency between them attained statistical significance, $P = 0.029$, suggesting that TNFR2 could be a new susceptibility gene for human narcolepsy. We further examined the relationship between the TNFR2 and TNF-a genes regarding the susceptibility to human narcolepsy. When the individuals possessing TNFR2-196R and TNF-a(-857T) alleles were investigated, it was found that the percentage of such individuals was significantly increased in the patients (16.1%) than in the controls (7.4%): the difference between them attained statistical significance, $P = 0.0096$. These data suggested the possibility that the TNFR2-196R and TNF-a(-857T) alleles had an additional effect on the susceptibility to human narcolepsy. Altogether, it may be possible that the TNF-a signal pathway participates in the pathogenesis and pathophysiology of human narcolepsy.

Identification of Candidate Genes for Th1 or Th2 mediated diseases using Functional Genomics. *R. Kinos, E. Ylikoski, S. Valkonen, H. Hamalainen, J. Savolainen, R. Lahesmaa.* Turku Centre for Biotechnology and Department of Pulmonary Diseases and Clinical Allergology, University of Turku, Turku, Finland.

The development and activation of CD4⁺ Th subsets with distinct patterns of cytokine production play important roles in infectious, allergic, and autoimmune diseases. Naive cord blood CD4⁺ human T cells can be polarized into Th1-like or Th2-like cells in vitro by culturing them in the presence of interleukin (IL)-12 or IL-4, respectively. Stat6 is a transcription factor critically involved in the IL4-receptor signaling and development of Th2 cells.

We have previously used this in vitro system and DNA-microarrays to identify 36 genes differentially expressed by Th1 or Th2 cells. Moreover, yeast-2-hybrid system has been used to identify 18 Stat6-interacting proteins from Th2 libraries. To elucidate the importance of this panel of known and novel candidate genes in the development of human immune mediated diseases, we have profiled their expression during lymphocyte development to Th1 or Th2 cells by TaqMan. As mutations in these marker genes might influence the level of gene expression and/or their encoding products, we have recently begun to screen these candidate genes for potential polymorphisms using DHPLC (Wave Technology). In parallel, the polymorphisms identified are being tested for potential linkage and/or associations with asthma and associated phenotypes.

A loss of function mutation of DNAS1L3 (leukocyte-Dnase) is found in the MRL model of systemic lupus erythematosus. *M.C. Schneider¹, A. Wilber¹, S.A. Abdullah¹, M. Lu².* 1) SIU Dept Ped, Div Gen/Metabol, SIU Sch Medicine, Springfield, IL; 2) Division of Research Urology, Brigham and Womens Hospital, Harvard Medical School, Boston, MA.

We find a partial loss-of-function mutation in the leukocyte-specific Dnase I-like-3 (DNAS1L3) gene in MRL strain, a murine model of polygenic systemic lupus erythematosus (SLE), a common multifactorial autoimmune disease characterized by antibodies against nucleosomal components. Recent experiments show mice deficient in deoxyribonuclease I (Dnase I) are highly predisposed to a phenotype similar to human SLE. The MRL-DNAS1L3 contains a non-conservative substitution (T89I), which decreases its deoxyribonuclease activity in in vitro functional assays by 54% ($p < .001$). In immunoblots of wild-type macrophage extracts using peptide-specific antisera, Dnas1l3 expression at baseline is low, but it is inducible by the PKC-activator and monocyte-differentiation agent, phorbol myristate acetate. In MRL, splenocytes and bone marrow-derived macrophages of 8-week-old mice show a 5-10X higher level of expression of Dnas1l3. In addition, similar over-expression of this enzyme is found in splenocyte and liver extracts of all other SLE-prone murine models, including BXSB, NZB, NZW, and NZB/W F1 mice, thus dysregulation of DNAS1L3 appears universally associated with polygenic models of lupus. Since this enzyme is likely involved in DNA scavenging by activated leukocytes, we predict deficiencies of DNAS1L3, as well as Dnase I, will be involved in the human susceptibility to SLE. We hypothesize the over-expression observed in murine lupus is in compensation for primary or secondary deficient activity. The abnormalities in lupus of this actin-resistant enzyme suggest a potential use of the enzyme in both therapy and diagnosis of the disease.

A replication study of the inflammatory bowel disease region on 6p. *B.M. Dechairo*^{1,2}, *N. Lench*¹, *D. van Heel*^{1,3}, *M. Edwards*¹, *P. Scambler*², *D.P. Jewell*³, *A.H. Carey*¹. 1) Gene Discovery, Oxagen Ltd., Abingdon, UK; 2) Molecular Medicine Unit, Institute of Child Health, University College of London, London, UK; 3) Gastroenterology Unit, Nuffield Department of Medicine, Radcliffe Infirmary, Oxford, UK.

Inflammatory Bowel Disease (IBD) includes ulcerative colitis and Crohn's disease, two chronic inflammatory diseases of the intestine. Aetiology is unknown but may involve a complex interaction between genetic susceptibility and environmental factors. Hampe et. al. (1999) reported significant evidence of linkage to IBD on 6p in a extension study following their genome scan using a cohort of 428 affected sib-pairs. We examined the same region in our population of 234 IBD European families containing 284 affected sibling pairs. Ten fluorescent labeled microsatellite markers were genotyped across the population. The markers spanned a 78 cM region. Order and distance between the markers was confirmed and refined by RH mapping using the Stanford G3 panel. Quality control of genotypes was conducted using Pedcheck 1.1 and CRI-MAP. There were 0.5% PCR fails, 0.15% CRI-MAP double recombinants, and 0.1% Pedcheck errors. Linkage analysis was performed using the MAPMAKER/SIBS functions implemented within GENEHUNTER 2.0. A multipoint LOD score of 3.0 was obtained approximately 15 cM centromeric to the peak marker from the study of Hampe et al. Our LOD score is greater than the threshold required to replicate significant linkage (Lander & Kruglyak, 1995) making this region highly likely to contain a gene conferring susceptibility to IBD.

The paternally expressed antisense *Ube3a*RNA is regulated by the Prader Willi syndrome Imprinting Center and appears to mediate imprinting of the *Ube3a*gene. C.I. Brannan, S.J. Chamberlain. Dept Molec Genetics/Microbiol, Univ Florida, Gainesville, FL.

Rougeulle *et al.*¹ reported a splice-variant of *UBE3A* which they determined to be a paternally expressed antisense transcript of the imprinted *UBE3A* gene. Rougeulle *et al.* suggested that the maternal-only expression of *UBE3A* may result from tissue specific expression of this paternally expressed antisense transcript. To test this hypothesis, we sought to determine if the antisense transcript regulates expression of *UBE3A* and if the antisense transcript is subject to regulation by the 15q11-q13 Imprinting Center (IC) using the mouse as a model system. We first confirmed that the murine *Ube3a* locus harbors a similar paternally expressed antisense transcript. Next, we determined that the antisense transcript was not expressed in mice harboring a paternally inherited PWS-IC deletion mutation, demonstrating that the antisense transcript was indeed regulated by the IC. Finally, we showed that in these PWS-IC deletion mice, the paternal allele of *Ube3a* is upregulated, suggesting that the antisense transcript negatively regulates the level of paternally-derived *Ube3a* mRNA. Together, these results strongly suggest that the imprinted expression of the *Ube3a* gene is an indirect consequence of expression of the IC-regulated antisense transcript.

¹Rougeulle, C., Cardoso, C., Fontes, M., Colleaux, L. & Lalande, M. An imprinted antisense RNA overlaps *UBE3A* and a second maternally expressed transcript. *Nature Genet.* **19**, 15-16 (1998).

Genetic complementation of Prader-Willi syndrome mice. *S.J. Chamberlain, C.I. Brannan.* Molecular Genetics and Micro., University of Florida, Gainesville, FL.

Prader-Willi syndrome (PWS) appears to be a contiguous gene syndrome resulting from mutation of two or more imprinted genes. Previously, we described a mouse model for PWS in which paternal transmission of a PWS Imprinting Center (PWS-IC) deletion mutation resulted in loss of local paternal gene expression. These PWS mice exhibit severe growth retardation, hypotonia and neonatal lethality. We have attempted to rescue these neonatal phenotypes via genetic complementation in an effort to functionally identify the causative genes in PWS. Our strategy has been to produce transgenic mice expressing one or more of the PWS candidate genes and then breed these transgenic mice to our PWS mouse model. Although this study will take some time to complete, we will present our preliminary results.

RNA-FISH detects changes in imprinted gene expression patterns as a result of 15q11-13 amplification or *MECP2* mutation. *L.B. Herzing*¹, *E.H. Cook, Jr.*², *D.H. Ledbetter*¹. 1) Dept of Human Genetics; 2) Dept of Psychiatry, Univ of Chicago, Chicago, IL.

15q11-13 abnormalities can result in Prader-Willi or Angelman Syndromes, Developmental Delay or Autism depending upon the parental origin of the affected chromosome. Many genes in this region are imprinted and neurally expressed. Using RNA-FISH, a powerful technique to analyze allele-specific expression of genes, we have previously shown that *UBE3A* is preferentially maternally expressed and therefore imprinted in tissues other than brain. Because UPD or trisomy can affect replication timing we wished to see whether regional amplification also variably influences the expression of imprinted genes. This could help explain the range of DD and autism spectrum disorders observed with identical ch15 duplications.

RNA was detected using cDNA and genomic probes; parent-of-origin determined by co-detection of genes with known imprint; and unamplified RNA signals confirmed by subsequent co-localization of genomic BAC probes. In fibroblasts from an autistic patient with mat dup(15q11-13), *UBE3A* expression levels from each of the duplicated maternal alleles was \geq than from the paternal allele in 82% of cells (vs. 89% in normals). However, 39% of pat dup(15) fibroblasts from the patient's phenotypically normal mother exhibited relatively higher levels of expression from one or both paternal alleles. This suggests that lack of biparental complement for an imprinted gene may result in loss of expression controls. Similarly, *UBE3A* expression is elevated from each allele of a large mat inv(dup) marker ch15, which has no endogenous, paternal complement chromosome with which to pair.

Other possible influences on imprinted expression in 15q11-13 are alterations in the mechanisms for reading and interpreting imprints, as illustrated by *MECP2* mutations in Rett Syndrome. Our preliminary findings in a Rett fibroblast line suggest that, indeed, these mutations affect imprinted expression patterns in 15q11-13, and work is underway to further characterize these patterns and determine whether these changes might account for the overlap between Autism and Rett phenotypes.

Long term survival of Prader-Willi syndrome mice. *T.A. Simon, S.J. Chamberlain, C.I. Brannan.* Molecular Genetics and Micro, University of Florida, Gainesville, FL.

Mice harboring a deletion of the paternally inherited Prader-Willi syndrome Imprinting Center (PWS-IC) do not survive beyond two weeks postpartum, thus limiting their potential use as a model system. Therefore, we sought to determine if PWS-IC deletion mice could be made to survive beyond this critical neonatal period by altering the strain background, using experienced mothers and/or reducing competition from wild-type siblings. Using a combination of these factors, we have successfully raised PWS mice that survive into adulthood. Both male and female surviving mice show reduced muscle mass and severe growth retardation compared to wild-type littermates. Studies are in progress to determine if the PWS mice are fertile and if they develop obesity.

Parent-of-origin specific histone acetylation at the imprinted *Snurf-Snrpn* locus in human and mouse. *S. Saitoh*¹, *M. Takahashi*¹, *T. Wada*¹, *T. Ohta*², *R.D. Nicholls*². 1) Dept. Pediatrics, Hokkaido Univ. Sch. Medicine, Sapporo, Japan; 2) Dept. Genetics, Case Western Reserve Univ., Cleveland, OH.

The molecular mechanism of gene regulation in genomic imprinting is hypothesized to involve chromatin based gene control. Chromosome 15q11-q13 is associated with Prader-Willi syndrome (PWS) and Angelman syndrome (AS), two classical imprinted disorders. Within the 2 Mb imprinted domain, the bicistronic *SNURF-SNRPN* locus appears to play a key regulatory role as it uniquely defines an imprinting center (IC) that controls the regulation of imprinting across the PWS/AS domain. To understand the potential role of histone acetylation in genomic imprinting, we have investigated the status of histone acetylation of the *SNURF-SNRPN* locus in human and mouse. Chromatin immunoprecipitation (ChIP) assays using anti-acetylated histone H3 and anti-acetylated histone H4 were performed to precipitate DNA associated with acetylated histones from lymphoblastoid cell lines from PWS and AS patients, and normal controls. For mice, we used fibroblasts from PWS and AS model mice carrying a 4 Mb deletion of the PWS/AS-homologous region on the paternal or maternal chromosome, respectively, and controls. Subsequent PCR using a series of primers covering the *Snurf-Snrpn* locus revealed hypoacetylation only in the CpG island of the maternally-derived inactive allele, which is completely methylated, whereas the unmethylated CpG island on the active paternal allele was associated with acetylated histones. In contrast, the body of the *Snurf-Snrpn* locus from each allele was equally acetylated for both human and mouse. We are currently extending the ChIP assay to other genes in the imprinted PWS/AS domain in both species. The co-localization of parent-of-origin specific histone acetylation and DNA methylation provides evidence that both epigenetic events are integrally involved in regulating genomic imprinting.

Absent imprinting effects on verbal IQ in girls with Turner syndrome. *J.L. Ross¹, H.F. Russell², T.J. Power², M.M. Mazzocco³, M. Muenke⁴, E. Pfendner¹, C.B. Boehm³, A.R. Zinn⁵*. 1) Dept. Pediatrics, Thomas Jefferson University, Philadelphia, PA; 2) Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA; 3) Kennedy Krieger Institute, Johns Hopkins University School of Medicine, Baltimore, MD; 4) Medical Genetics Branch, National Human Genome Research Institute, NIH, Bethesda, MD; 5) McDermott Center for Human Growth and Development, University of Texas Southwestern Medical School, Dallas, TX.

BACKGROUND: Turner syndrome (TS) is the complex phenotype of human females with complete or partial absence of the second X chromosome. TS females have normal verbal abilities and specific deficits in visual-spatial abilities. The underlying etiology may be genetic, hormonal, or both. An additional genetic mechanism, imprinting, may also contribute. According to Skuse et al.(1997), TS patients whose single X was maternal had significantly poorer verbal skills, higher-order executive function, and socio-behavioral skills than patients whose single X was paternal in origin. **AIM:** To evaluate the contribution of imprinting to cognitive performance (IQ) in TS females.

SUBJECTS/METHODS: Eighty three 45,X subjects (ages 7 to 41 years) had either the WISC-R, the WISC-III, or the WAIS-R. The parent of origin of the intact X chromosome was determined by comparing the patient and parental alleles for a number of highly polymorphic markers along the length of the X chromosome. **RESULTS:** Analysis of the entire 45,X group revealed a higher Verbal IQ (102 ± 13) than Performance IQ (85 ± 13) with a Full Scale IQ of 93 ± 13 overall. The two groups (X_{pat} and X_{mat}) were well matched for age (13.8 ± 3.1 , $n=24$ and 13.2 ± 5.6 , $n=59$, $p=0.51$) and socioeconomic level. The IQ results were similar for the subjects with the paternally versus maternally derived X chromosome: VIQ: 100 ± 13 vs 103 ± 13 ($p=0.40$); PIQ: 82 ± 14 vs. 86 ± 13 ($p=0.28$); and FSIQ: 91 ± 14 vs. 94 ± 13 ($p = 0.29$). **CONCLUSION:** In contrast to previously published studies, no imprinting effect on verbal abilities or cognitive performance (IQ) was observed in TS subjects.

TDT Analysis of candidate gene polymorphisms for schizophrenia. *V.L. Nimgaonkar¹, K.V. Chowdari¹, F. Zhang¹, A.L. Butler¹, J. Wood¹, I.I. Gottesman².* 1) Dept Psychiatry, Rm 443, Univ Pittsburgh, Pittsburgh, PA. E-mail vishwajitnl@msx.upmc.edu; 2) Dept of Psychology and Pediatrics, University of Virginia, Charlottesville, VA.

Association studies of schizophrenia using unrelated controls are prone to artifacts due to population sub-structure. Family based analyses such as the Transmission Disequilibrium Test (TDT) are therefore currently preferred. However, the TDT imposes restrictions regarding family structure and parental genotype. This limits its usefulness, as shown in our ongoing studies. We evaluated 168 simplex families consisting of patients with schizophrenia (DSM IV criteria) and available parents at the following candidate genes / regions: cPLA2, DRD1, DRD3, hSKCa3, DCP1, CHRNA7, D22S278 and chromosome 6p21.3. We did not detect significant preferential transmission of any allele from heterozygous parents to affected offspring. The requirement of heterozygous parents for the TDT, as well as exclusion of families with single homozygous parents resulted in substantial numbers of exclusions. For example, only 58 parental genotypes were included when a restriction fragment length polymorphism (RFLP) at DRD3 was investigated using the computer software MCETDT. Haplotype based TDT analysis using 3,4, a short tandem repeat polymorphism (STRP) localized <1 kb from the DRD3 RFLP did not increase informativeness. It may be advisable to use the TDT in conjunction with analysis using unrelated controls. This is illustrated with respect to DCP1, for which we examined a bi-allelic polymorphism simultaneously among two independent sets of unrelated controls.

Linkage disequilibrium between the NMDAR1 gene and Bipolar Disorder. *E. Mundo¹, S. Tharmalingam¹, M. Walker¹, A. Bolonna², R.W. Kerwin², F. Macciardi¹, J.L. Kennedy¹.* 1) Neurogenetics Section, CAMH, University of Toronto, Toronto, Ontario, Canada; 2) Department of Psychological Medicine, King's College of London, University of London, U.K.

The glutamate system is implicated in the pathogenesis of major psychoses. A glutamatergic deficiency model has been proposed for schizophrenia (Carlsson et al, 1999), and the drug valproate (commonly used to treat Bipolar illness) has been found to stimulate glutamate release, which activate the N-Methyl-D-aspartate receptor (NMDAR) (Dixon and Hokin, 1997). This last finding suggests a role of NMDAR in the pathogenesis of Bipolar Disorder (BP). The key subunit of the NMDAR, named NMDAR1, is encoded by a gene located on chromosome 9q34.3 (Collins et al, 1993). For this gene two silent polymorphisms in the coding region and five in non-coding regions have been identified (Bolonna et al, 1999). We investigated for the presence of linkage disequilibrium between the NMDAR1 gene (the 1001-G/C and the 1970-A/G polymorphisms) and BP. Two hundred and seventy BP I or BP II probands with their living parents were studied. Diagnoses were assessed by a structured interview for DSM-IV (American Psychiatric Association, 1994) (SCID-I). Genotyping data were analyzed using the Transmission Disequilibrium Tests (TDT) (Spielman et al, 1993). Seventy-three triads had heterozygous parents for the 1001-G/C polymorphism, and 174 triads had heterozygous parents for the 1970-A/G polymorphism. These triads were suitable for the final analyses. For the 1001-G/C polymorphism we found a preferential transmission of the G allele to the affected subjects (chi-square=5.232, df=1, p=.02) while the two alleles of the 1970-A/G polymorphism were transmitted with similar frequency to the probands (chi-square=1.332, df=1, p=ns). If the results on the 1001-G/C polymorphism are replicated there will be important implications for the involvement of the NMDAR1 gene in the pathogenesis of BP.

Disequilibrium analysis of the SNAP25 gene in schizophrenia and bipolar disorder. *T.A. Klempan¹, A. Wong², T. Armstrong¹, N.A. King¹, C.L. Barr³, C. Pato⁴, M. Pato⁴, M.H. Azevedo⁴, F. Maciardi¹, J.L. Kennedy¹.* 1) Neurogenetics, CAMH, Toronto, Ontario, Canada; 2) Molecular Neurobiology, CAMH, Toronto, Ontario, Canada; 3) Department of Psychiatry, The Toronto Western Hospital, Toronto, Ontario, Canada; 4) Department of Psychiatry, SUNY at Buffalo, NY.

Synaptosomal-associated protein of 25 kDa (SNAP-25) is a highly conserved member of the exocytotic fusion complex (T-SNARE) which regulates vesicular trafficking and neurotransmitter release in various populations of cortical neurons and neuroendocrine cells. Its expression is associated with regions of synaptic plasticity, promoting neurite extension during synaptogenesis and neuron repair. Several studies have implicated SNAP-25 in the etiology of schizophrenia based upon altered levels of brain expression in humans and rodent models, and altered CSF protein levels. To examine the potential role of this candidate in the pathogenesis of schizophrenia and bipolar disorder, we have studied DNA sequence variants in the 3' untranslated region that alter restriction sites for the enzymes MnlI and DdeI. Genotyping of these variants was carried out in a sample of 202 small nuclear families with bipolar disorder (parents and proband) and 90 nuclear schizophrenia families. The transmission of both alleles and haplotypes of these polymorphisms to affected offspring was investigated using the transmission disequilibrium test (TDT). Unequal transmission of alleles was witnessed in the families affected with bipolar disorder (allelewise $c^2=3.65$, 1df, $p=0.056$ for DdeI; haplotypewise $c^2=4.85$, 1df, $p=0.028$ for 1.1 haplotype; global $c^2=8.00$, 3df, $p=0.046$ for all haplotypes). The results of TDT analysis in schizophrenia families of mixed ethnic background are nonsignificant ($c^2=1.05$, 1df, $p=0.305$ for DdeI). This finding of linkage disequilibrium supports the hypothesis that either SNAP25 or a nearby locus may play a role in the development of neuropsychiatric disease and is being further studied through analysis of a third SNAP25 polymorphism.

Investigation of NMDA1 and GRIN2B Receptor Polymorphisms in the Pathogenesis of Schizophrenia. L.

Martucci¹, A. Wong², J. Trakalo¹, T. Cate¹, A. Ambrosio⁴, M. Pato³, C. Pato³, M.H. Azevedo³, C. Olivera⁴, F. Macciardi¹, J. Kennedy¹. 1) Neurogenetics, CAMH, Toronto, Ontario, Canada; 2) Molecular Neurobiology, CAMH, Toronto, Ontario, Canada; 3) Department of Psychiatry, SUNY, Buffalo, NY, USA; 4) Centro de Neurociencias de Coimbra, Departamento de Zoologia/Fac. Medicina, Universidade de Coimbra, Portugal.

The different subtypes of N-Methyl-D-Aspartate Glutamate receptors (NMDAR) act in the CNS as regulators of the release of neurotransmitters such as dopamine, noradrenaline, acetylcholine and GABA. This suggests glutamatergic system dysfunction may play a role in the pathogenesis of schizophrenia, and animal models are currently under investigation. We have focused on the study of two polymorphisms in the NMDAR1 gene, and of one polymorphism in the NMDAR2B receptor subunit gene (called GRIN2B for glutamate receptor, ionotropic, N-methyl-aspartate 2B). All the polymorphisms are single base changes: NMDAR1/1 is a G/C substitution localized on the 5' untranslated region (UTR); NMDAR1/10 is an A/G substitution localized in exon 6; and there is a T/G substitution in the 3'UTR of the GRIN2B gene. The minor allele frequency in our sample was calculated to be 0.05, 0.2 and 0.4 respectively. We genotyped 41 nuclear families, and tested the hypothesis that NMDAR and GRIN2B polymorphisms were associated with schizophrenia using the Transmission Disequilibrium Test. No association was found: NMDAR1/1, $c^2 = 0.333$, $p=0.57$; NMDAR1/10, $c^2 = 0.429$, $p = 0.51$; GRIN2B, $c^2 = 1.19$, $p= 0.2753$. Polymorphisms NMDAR1/1 and NMDAR1/10 were also tested in our laboratory on a sample of 90 Portuguese schizophrenia trios; again, no association was found: $c^2=0$, $p=1$ for NMDAR1/1; $c^2=0.744$; $p=0.388$ for NMDAR1/10. We are currently collecting additional samples to increase the power of the analyses.

Association analysis of the DRD4 and DAT1 genes in a Dutch ADHD population. *R.J. Sinke¹, S.C. Bakker¹, N. Oteman¹, E. van der Meulen², J.K. Buitelaar², P.L. Pearson¹.* 1) Department of Medical Genetics, University Medical Center Utrecht, The Netherlands; 2) Department of Child Psychiatry, University Medical Center Utrecht, The Netherlands.

Attention deficit hyperactivity disorder (ADHD) is one of the most frequent causes of childhood disruptive behaviour, affecting 3-5% of school-age children. Family, adoption and twin studies have shown strong evidence for the involvement of genetic factors in the susceptibility to develop ADHD. Accordingly, it is estimated that the heritability of ADHD is 60 to 80%. Genetic research aimed at the identification of the genes involved in ADHD is limited and has, so far, focused on the analysis of several candidate genes. Recent studies indicate associations of ADHD with genes involved in the regulation of dopamine neurotransmission. A variety of investigators have reported positive findings for the dopamine DRD4 receptor gene and the dopamine transporter gene (DAT1). The aim of this study is to replicate the reported associations, i.e. the 7-repeat allele of the DRD4 gene and the 10-repeat allele of the DAT1 gene. We examined the VNTR polymorphisms using the transmission disequilibrium test (TDT) in 81 families containing at least one child diagnosed with ADHD. TDT analysis showed positive association for the 10-repeat DAT1 allele and no significant association for the 7-repeat DRD4 allele. These findings were further validated by extending our studies with haplotype analysis of additional polymorphisms from these gene regions.

Follow-up Analysis of the Chromosome 19q13 Region in Multiple Sclerosis with Single-Nucleotide

Polymorphisms. *J.B. Rimmeler¹, S. Schmidt¹, F. Boylston¹, A. Rogala¹, J.M. Vance¹, E. Lai⁴, E. Martin¹, J.R. Oksenberg³, S.L. Hauser³, R.R. Lincoln³, L.F. Barcellos³, M.A. Pericak-Vance¹, J.L. Haines², Multiple Sclerosis Group⁵.* 1) Duke University Medical Center, Durham, NC; 2) Vanderbilt University Medical Center, Nashville, TN; 3) University of California, San Francisco, CA; 4) Glaxo-Wellcome Research & Development, Research Triangle Park, NC; 5) Multiple Sclerosis Genetics Group.

Multiple sclerosis (MS) is an autoimmune neurological disorder with a complex and poorly understood etiology. Genomic screens to identify susceptibility genes for MS have been performed, however, little agreement between studies was found. Aside from the MHC, the only other region positive in all screens was the chromosome (chr) 19q13 region near the APOE gene. Further detailed analysis of this region in outbred multiplex families (MSGG, 2000; Barcellos et al., 2000) and a homogeneous population in San Marino (Haines et al., 2000) supports a 19q13 susceptibility locus. To thoroughly examine 19q13 and to investigate the usefulness of single-nucleotide polymorphisms (SNPs) as the acclaimed next generation of genomic markers, the MSGG ascertained 428 outbred families for linkage disequilibrium analysis. The dataset includes 157 multiplex and 271 simplex families. We designed an approach that efficiently uses all available multiplex and simplex families, a moderate-throughput oligonucleotide ligation assay for SNP genotyping, and newly developed statistical approaches that combine all available families into valid tests of association. To validate this approach, we are typing 15 previously described SNPs near APOE and are analyzing them using the pedigree disequilibrium test (PDT) and the haplotype association analysis program Transmit. Preliminary results of the first 2 SNPs have been analyzed. Global statistics from the PDT as well as single-locus and two-locus Transmit analyses generated non-significant ($p=0.39$ to 0.96) results. This approach will now be used to genotype an additional 13 SNPs in 19q13. The comparison of SNP results to microsatellite results will help to clarify the true value of SNPs in the search for genes in complex diseases.

Genome scan of autistic spectrum disorders in the Finnish population. *M.P. Auranen^{1,2}, T. Ylisaukko-Oja¹, R. Vanhala³, T. Nieminen³, T. Varilo¹, L. Peltonen^{1,2,4}, I. Järvelä^{1,2}.* 1) Department of Human Molecular Genetics, National Public Health Institute, Helsinki, Finland; 2) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 3) Unit of Child Neurology, Hospital for Children and Adolescents, Helsinki, Finland; 4) Department of Human Genetics, UCLA School of Medicine, Los Angeles, CA.

Autism spectrum disorders (prevalence 5-8/1000), infantile autism and Asperger syndrome are characterized by severe social, language and communication deficits. Based on epidemiological and family studies the role of genetic component is strong; however the etiopathogenesis is unknown and no curative treatment is available. We have analyzed ten chromosomal regions (1p, 4p, 6q, 7q, 13q, 15q, 16p, 17q, 19q and 22q) that have potentially been linked to autism in 17 Finnish families with multiple affected individuals suffering from autistic spectrum of disorders. No evidence for linkage or association was achieved using pairwise linkage and sib-pair analysis (Auranen et al., in press). We have conducted a primary genome screen in 22 multiplex Finnish families with 51 affected individuals. To maximize the genetic and cultural homogeneity, the families were chosen from Central Finland where the majority of the ancestors originate. 390 microsatellite markers were genotyped with ABI 377 Sequencer with intermarker distance about 10cM. We identified four loci in families with infantile autism: D1S1675 (Zmax 1.4), D3S3053 (Zmax 2.0), D3S2418 (Zmax 2.0) and D6S305 (Zmax 1.5). These loci are currently under fine mapping. Since autism and Asperger syndrome are cosegregating in the same families it is possible that same not yet known loci contribute to both diseases. From our collection of over 100 families with autistic spectrum of disorders the mode of inheritance seem to be different in families with infantile autism or Asperger syndrome, the former showing mainly autosomal recessive and the latter autosomal dominant inheritance. To add our understanding about susceptibility gene loci and genes underlying Asperger syndrome we are currently performing candidate gene analysis in 14 megafamilies with Asperger syndrome.

Family Based Transmission Disequilibrium Test Reveals Linkage Between an SP-B-linked Locus and RDS; SP-B-linked Alleles are Susceptibility or Protection Factors for RDS. *J. Floros¹, R. Fan², X. Guo¹, A. Matthews², J. Luo¹*. 1) Department of Cell & Molecular Physiology, The Pennsylvania State Univ ColMed, Hershey, PA; 2) Department of Health Evaluation Sciences, The Pennsylvania State University, Hershey, PA.

Respiratory distress syndrome (RDS) is a disease occurring in the prematurely born infant. Both low levels of surfactant protein B (SP-B) and specific SP-B alleles have been associated with RDS. In the present study we performed family based transmission disequilibrium test (TDT) analysis to determine whether the SP-B locus or SP-B-linked loci are linked to RDS and whether certain alleles are susceptibility or protective factors for RDS. Eight SP-B or SP-B-linked marker loci were tested for linkage with RDS. We genotyped 32 nuclear families for these alleles. Each family had at least one affected child. The results showed that the transmission of allele 7 of marker D2S2232 and allele 4 of marker D2S388 is significantly increased ($p=0.025$ and $p=0.008$, respectively) in the child with RDS. However, allele 5 of marker D2S2232, allele 2 of marker D2S388, and allele 8 of marker AAGG either failed to be transmitted or their transmission to the affected child was significantly reduced ($p=0.046$, 0.035 , and 0.035 , respectively). These data indicate that the SP-B linked loci are linked to RDS. Moreover, based on the frequency of transmission, allele 7 of marker D2S2232 and allele 4 of marker D2S388 may be viewed as susceptibility factors, whereas, allele 5 of marker D2S2232, allele 2 of marker D2S388, and allele 8 of marker AAGG can be viewed as protective factors for RDS. This work was supported by NIH R37 HL34788 and GCRC.

The PPAR-g 2 Pro12Ala variant: association with type 2 diabetes, trait differences, and interaction with the β_3 -adrenergic receptor. *M.R. Erdos¹, J.A. Douglas², R.M. Watanabe², A. Braun³, P. Oeth³, C. Johnston³, K. Mohlke¹, T. Valle⁴, T.A. Buchanan⁵, R.N. Bergman⁵, F.S. Collins¹, M. Boehnke², J. Tuomilehto⁴, and The FUSION Study Group^{1,2,3,4,5}.* 1) National Human Genome Research Institute, NIH, Bethesda, MD; 2) University of Michigan, Ann Arbor, MI; 3) Sequenom Industrial Genomics, San Diego, CA; 4) National Public Health Institute, Helsinki, Finland; 5) University of Southern California, Los Angeles, CA.

Recent studies have identified a common proline-to-alanine substitution (Pro12Ala) in the peroxisome proliferator-activated receptor-g 2 (PPAR-g 2), a nuclear receptor that regulates adipocyte differentiation and possibly insulin sensitivity. The Pro12Ala variant has been associated in some studies with type 2 diabetes and/or diabetes-related traits. We examined this variant in 1618 Finnish subjects: 785 index cases with type 2 diabetes, 193 non-diabetic spouses, 420 non-diabetic offspring, and 220 elderly non-diabetic controls. The frequency of the Pro12Ala variant was significantly lower in diabetic compared to non-diabetic subjects (0.15 versus 0.21; $p < 0.001$). We also compared diabetes-related traits between subjects with and without the Pro12Ala variant within clinical subgroups. Among subjects with type 2 diabetes, the variant was associated with greater weight gain relative to weight at age 20 ($p = 0.023$) and lower triglycerides ($p = 0.033$). The variant was associated with higher diastolic blood pressure (DBP) only for severely obese ($\text{BMI} > 40 \text{ kg/m}^2$) diabetic subjects. A similar association with DBP and systolic blood pressure (SBP) was also observed among very obese non-diabetic offspring. In non-diabetic spouses, the variant was associated with higher fasting insulin ($p = 0.033$), SBP ($p = 0.021$), and DBP ($p = 0.045$). Among type 2 diabetic subjects also genotyped for the Trp64Arg variant of the β_3 -adrenergic receptor (ADRB3) ($n = 468$), presence of the Pro12Ala variant and the Trp64Arg variant was associated with higher waist-to-hip ratio ($p = 0.025$), lower HDL cholesterol ($p = 0.025$), higher triglycerides ($p = 0.043$), and higher SBP ($p = 0.030$). These findings support a role for the PPAR-g 2 Pro12Ala variant in the etiology of type 2 diabetes.

No evidence of linkage with chromosome 1 markers for age-related maculopathy phenotypes: The Beaver Dam Eye Study. *S.K. Iyengar¹, J.H Schick¹, B. Klein², R. Klein², B. Fijal¹, K. Jacobs¹, R.C. Elston¹.* 1) Epid/Biostat, R203A Rammelkamp, Case Western Reserve Univ, Cleveland, OH; 2) Department of Ophthalmology and Visual Sciences, Univ of Wisconsin Medical School, Madison, WI.

Age-related maculopathy is a multifactorial disorder caused by degeneration of the macula in the retina, leading to loss of peripheral central vision. Previously, two regions on chromosome 1, the ABCR gene (1p22-p21), and a 12.2 cM region on 1q25-q31 have been associated/linked with age-related maculopathy. We analyzed 2 markers (D1S406 & D1SS236) spanning a 2cM region around the ABCR gene and 4 markers (D1S466, D1S202, D1S2625 & D1S413) encompassing a 12.2 cM region on 1q25-q31 in age-related maculopathy families from a community sample in Beaver Dam, Wisconsin. One hundred families (N=325 subjects) with at least 2 affected sibs, consisting of 257 sib pairs, were genotyped. In families where genotyping inconsistencies could not be resolved after two rounds, relationships were reclassified with two hundred markers from a genome scan for age-related maculopathy (in progress). SIBPAL2 (S.A.G.E.) was used for single-point and multi-point linkage analyses of the age-related maculopathy quantitative trait. Linkage analyses were adjusted for covariates: age, age², smoking status, drinking habits and diabetes. To ensure that presence of diabetes did not confound the interpretation of linkage results for age-related maculopathy, diabetes alone was also analyzed as an outcome. After adjusting for covariates, the results of the single-point linkage analysis for D1S413 were significant at $p=0.03$ for the age-related maculopathy phenotype. However, multi-point linkage analyses with all four 1q25-q31 makers were not significant ($p > 0.05$). Our results did not suggest significant major gene effects for age-related maculopathy at the ABCR gene, or other markers on chromosome 1. Results are currently being verified in an independent sample of large families from Beaver Dam, Wisconsin. This research was supported by NIH grant 1R01EY10605.

Identification of a Novel Rest:Activity Mutant in the Mouse, Earlybird *Earlybird*. D.J. Kapfhammer^{1, 2}, G. Leach^{1, 2}, O. Valldares^{1, 2}, S. Veasey^{3, 4}, M. Bucan^{1, 2}. 1) Center for Neurobiology and Behavior, University of Pennsylvania; 2) Department of Psychiatry, School of Medicine, University of Pennsylvania; 3) Center for Sleep and Respiratory Neurobiology, University of Pennsylvania; 4) Department of Medicine, School of Medicine, University of Pennsylvania.

Our laboratory is conducting a dominant screen for single gene mutations affecting several endophenotypes of human psychiatric disorders that can be modeled in the mouse. These include screens for abnormal prepulse inhibition and habituation of acoustic startle (to measure sensorimotor gating, an endophenotype of schizophrenia), zero maze (to measure anxiety-like behaviors), and rest:activity behavior as assayed by wheel-running activity (potentially indicative of an underlying sleep and/or affective disorder). To date, several mutants have been identified. One mutant, Earlybird (Ebd), exhibits an abnormally shortened period of activity in constant darkness; Earlybird heterozygotes display a period between 22.5 and 23.1 hours (compared to 23.7 hours for wild-type C57BL/6J mice), while the period of Ebd/Ebd homozygotes ranges from 22.0 to 22.5 hours. To investigate whether the short circadian period of activity in Earlybird mice generalizes to other parameters under circadian regulation, the pattern of sleep states (waking, NREM, and REM) was assessed in Ebd/Ebd, Ebd/+ and wild-type mice by monitoring EEG and EMG activity. Although no effect of genotype on baseline amounts of waking, NREM or REM was observed, tDD of these sleep states is shortened in Ebd/Ebd and Ebd/+ mice in comparison to wild-type littermates. Earlybird maps to the proximal portion of mouse chromosome 8 in a region syntenic to human 19p13.3. The recent completion of the human chromosome 19 draft sequence provides a powerful tool for identifying potential candidate genes within this 4.5cM candidate region. Among these include Npy1r (neuropeptide Y receptor Y1), Jund1 (Jun protooncogene related gene-d1), Pik3r2 (phosphatidylinositol 3-kinase regulatory subunit polypeptide 2), Mel (ras-associated protein RAB3), and Scl18a1 (vesicular monoamine transporter 1). The potential relevance of these genes to the Ebd mutation is currently under investigation.

A model for the genetic etiology of autism spectrum disorders involving alleles at maternal and fetal

susceptibility loci. *J.J.A. Holden¹, C.K. Schutz^{1, 2}, P.D. Robinson², D. Polley^{1, 2}, F. Macciardi³, B.N. White².* 1) Queen's University and Ongwanada, Kingston, ON, Canada; 2) McMaster University, Hamilton, ON, Canada; 3) Centre for Addiction and Mental Health, Toronto, ON, Canada.

Autism spectrum disorders (ASDs) are characterized primarily by deficits in social interaction and communication, resistance to change, and repetitive stereotypic behaviours and activities. Twin and family studies support a strong role for genetic factors in the etiology of ASDs. The mode of inheritance of ASDs is unclear, but the unusual sex ratio and a relatively low recurrence risk to sibs are indicative of a complex genetic etiology, likely involving multiple loci and causal heterogeneity. We are using the affected sib-pair method and TDT to examine the role of candidate genes in the etiology of ASD. Based on our findings at the DBH, MAOA, and biogenic amine transporter loci, we propose that at least some cases of ASD result from abnormal monoamine neurotransmitter levels in maternal serum resulting from functional variations in the dopamine beta hydroxylase (DBH) and monoamine oxidase A (MAOA) genes. We suggest that this maternal effect, which is dependent on the mother's genotype at the DBH and/or MAOA genes, creates a potentially damaging uterine environment in which the fetal brain may not develop normally, resulting in susceptibility to ASD. We propose that the maternal effect is modulated by fetal-specific factors, which appear to include fetal genotypes at the monoamine (serotonin, dopamine, and norepinephrine) transporter genes, but may also include such elements as fetal gender and birth weight. The severity of the maternal effect may also be modulated by other biochemical and genetic factors, as well as by environmental influences. This maternal effect/ fetal susceptibility model explains most of the unusual genetic features of autism spectrum disorders.

Testing for maternal and fetal effects in autism. *P. Szatmari¹, M.B. Jones², L. Zwaigenbaum¹, R.M. Palmour³, J. Meji³.* 1) Dept Psychiatry, McMaster Univ, Hamilton, ON., Canada; 2) Pennsylvania State University College of Medicine, Hershey, Penn; 3) Dept Psychiatry, McGill University, Montreal, Que, Canada.

Autism is a complex genetic disorder caused by multiple genes and perhaps gene environment interactions. Cook et al. (1997) reported evidence of linkage and association between a common polymorphism in the serotonin transporter (5HTT) gene and autism, but Klauck et al. (1997) reported a significant association with the alternate allele. Both studies were conducted in simplex (SPX) families, i.e. those with one affected child. Maestrini et al. (1999) reported no association at this locus in a sample of largely multiplex (MPX) families with at least two affected children so the importance of these findings is unclear. Based on previous evidence of reduced serum dopamine beta hydroxylase (DBH) levels in parents of autistic children (Lake et al. 1977), we hypothesized that autism may be caused by an interaction between the maternal genotype at the DBH locus (leading to altered levels of catecholamines in utero) and the fetal genotype at the 5HTT locus. None of the studies outlined above attempted to replicate their findings in both MPX and SPX families simultaneously or attempted to systematically search for maternal effects in autism in interaction with susceptibility genes. We extended the log linear approach to case-parent triads first outlined by Weinberg et al. (1998) to test for maternal effects and fetal susceptibility genes in 39 informative MPX and 60 SPX families, all diagnosed by best-estimate using both structured tools and medical records with some type of autistic spectrum disorder. We found a significant fetal effect for DBH in MPX sibships ($p < .001$) but not in SPX sibships, a result probably due to chance. No maternal effects were significant and no association with 5HTT was found. Although we obtained non-significant results, the methodology employed appears useful in searching for maternal and fetal effects in autism and may be extended to other complex genetic disorders that potentially involve a maternal effect.

Screening EDN-1 and LHX-8 for mutations associated with nonsyndromic cleft lip and palate. *R. Schultz¹, A. McColley¹, N. Malik², H. Westphal², J. Murray¹.* 1) Dept Genetics, Univ Iowa; 2) Nat'l Instit Child Health and Human Devel, Bethesda, MD.

Cleft lip and palate is a congenital anomaly present in about 1/1000 live births. 30% of cases are syndromic, caused by Mendelian disorders, chromosomal anomalies, and teratogens, e.g. The remaining 70% are nonsyndromic; the affected individual has no other abnormalities. Studies showed linkage of NS CL/P to 6p23, which contains the candidate gene endothelin-1. The EDN-1 knockout mouse has craniofacial abnormalities. All 5 exons and the intron-exon boundaries of EDN-1 were screened for mutations by SSCP analysis. Filipino probands with NS CL/P and both unaffected parents were screened for mutations. An SSCP variant was found in exon 2, a G to A change at bp 426 of the coding sequence. This change is a silent change, making it unlikely to be a disease-causing mutation. The p-value for the transmission disequilibrium test was 0.1243. In exon 1, a variant was identified as an A insertion at bp 204, before translation start. The p value for the TDT was 0.7935. The p values for the TDT are not significant, so it is unlikely that either variant is disease-causing. LHX8 is a LIM homeodomain protein. FISH and radiation hybrid mapping place Lhx8 in 1p32. The Lhx8 knockout mouse has cleft palate without other craniofacial abnormalities. In situ studies in wild-type mouse embryos show Lhx8 is expressed in the maxillary processes and palate mesenchyme during palatal shelf outgrowth, elevation, and fusion. LHX8 has 9 exons. Part of the 5'UTR, all of exons 1-3, and the intron-exon boundaries were screened by SSCP analysis for mutations in Iowa probands with cleft palate and both unaffected parents. One variant has been identified, a A to C change at bp 27 of intron 1. 24 families have been screened for this variant. The p-value for the TDT is 0.7480. The p-value is not significant, and the variant is in an intron and does not interrupt a splice site, which makes this change more likely to be a neutral polymorphism than a disease-causing mutation. More families will be screened for this variant to increase the sample size. Exons 4-9 will be screened for SSCP variants.

A genome-wide scan for low HDL-cholesterol in genetically isolated Finnish families with premature coronary heart disease. *A.M Soro*^{1,2}, *H. Lilja*³, *P. Pajukanta*¹, *K. Ylitalo*², *I. Nuotio*¹, *J.S.A. Viikari*⁴, *M-R. Taskinen*², *L.*

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Low high-density lipoprotein -cholesterol (HDL-C) level is a well- established risk factor for premature coronary heart disease (CHD). To elucidate the genetic background of low HDL-C and premature CHD in the genetically isolated Finnish population, we carried out a genome- wide search for predisposing loci. A total of 25 well-documented Finnish low HDL pedigrees were collected. Inclusion criteria for the probands were as follows: Age of 30-60 years, CHD and HDL-C level below the age-sex specific 10th Finnish population percentile. At least one first-degree relative fulfilled the same strict criteria for HDL. A two-stage genome-wide scan was performed. In stage 1, we genotyped a total of 176 individuals, from 20 well-defined low HDL families with 357 microsatellite markers with an average marker interval of 8.5 cM. We applied both parametric linkage and nonparametric affected sib-pair (ASP) analysis to identify interesting chromosomal regions for fine mapping. In stage 2, the regions with positive results (LOD score greater than 1.0) were covered with denser marker maps. Additional families were genotyped together with the original sample set with the denser marker maps to specify the significance of the preliminary findings. Out of 11 chromosomal regions identified in stage 1, seven chromosomal regions gave further support for linkage by resulting in LOD scores greater than 2.0 in two-point linkage analysis: Chromosomes 1, 2, 3, 6, 8, 16 and 20. Maximum lod scores of 2.69 and 2.52 were obtained on chromosomes 2 and 16. Further studies are required to verify the significance of these novel loci in the HDL genetics.

Polymorphisms of the Flavin Containing Monooxygenase 3 (*FMO3*) gene predispose to early onset Essential Hypertension in French Canadians. *E.P. Treacy*^{1,2}, *D.M. Lambert*¹, *B.R. Akerman*¹, *H. Yang*¹, *S. Ryan*¹, *R. Platt*¹, *J. Genest*³, *D. Gaudet*⁴, *P. Hamer*⁵. 1) Montreal Childrens Hospital, McGill University Hospital Complex (MUHC), McGill University, Montreal, Canada; 2) National Centre for Inherited Metabolic Diseases, The Childrens Hospital, Dublin, Ireland; 3) MUHC, McGill University, Montreal, Canada; 4) Hospital Complex of Sagamie, Quebec, Canada; 5) Hospital Center of the University of Montreal (CHUM), Montreal, Canada.

Interindividual variation in blood pressure is genetically determined resulting from the interaction of susceptibility alleles and environmental exposures. We previously documented that rare mutations of the *FMO3* gene (a phase 1 xenobiotic detoxicating gene) cause the inborn error of metabolism Trimethylaminuria and may predispose affected individuals to essential hypertension (HBP) and abnormal catecholamine metabolism (Treacy et al, 1998). We subsequently characterised three polymorphisms of this gene, (E158K (c.488 G-A), E308G (c.923 A-G) and V257M (c.769 G-A)). Haplotypes were phased in 60 extended families from Quebec. Of 8 possible haplotypes, 6 were observed. Our *in vivo* and *in vitro* data indicate that these polymorphisms in particular haplotypes confer functional pharmacogenetic effects. We now report a case control study of the association between variant *FMO3* haplotypes and early onset HBP (< 55 years) in a Quebec French Canadian population. The distribution of haplotypes was determined between (1) HBP probands (n = 60), (2) HBP relatives (n = 229), (3) non-HBP control relatives (n = 145) and (4) unrelated non-HBP French Canadian controls (n = 133). There was a significant difference noted between the distribution of haplotypes between groups (1) and (4) (p = 0.026). The presence of one variant haplotype in individuals conferred a relative risk of HBP of 1.22 (95% CI: 1.0 - 1.5), the relative risk was 1.97 (95% CI: 1.01 - 3.84) in individuals who carry two variant haplotypes compared to those homozygous for the wild type haplotype. These data suggest that the above common polymorphisms of the *FMO3* gene in the presence of adverse environmental exposures may increase the risk of individuals in Quebec to early onset HBP.

Evidence of Susceptibility Loci in Genome Wide Searches of Familial Idiopathic Scoliosis. *C.A. Wise¹, J.A. Herring¹, S. Shoemaker¹, J.D. Gillum¹, H.C. Gunn¹, M. Lovett², L.B. Bennett², A.M. Bowcock².* 1) Research Dept, Texas Scottish Rite Hosp, Dallas, TX; 2) Washington University School of Medicine, St. Louis, MO.

Idiopathic scoliosis (IS) is defined as lateral curvature of the spine of unknown etiology. The frequency of IS in human populations is high, with estimated occurrences of 1.5-3%, 0.3-0.5%, and 0.2-0.3% for curves over 10, 20, and 30 degrees, respectively. If left untreated IS can be fatal secondary to cardio-pulmonary compromise. Approximately 30% of IS patients report a family history of the disease. IS in general is a complex disorder, although some families with a Mendelian inheritance pattern have been described. We are ascertaining and sampling such multiplex IS kindreds and combining stringent diagnostic criteria with conservative methods of genetic analysis to positionally clone genes predisposing to the disease. Probands having clinically relevant IS (50 degree Cobb angle or greater) were identified. Positive diagnoses required a minimum curve of 15 degrees confirmed from standing posteroanterior radiographs. We have completed genome-wide scans in three extended kindreds (24 affected members of 50 sampled) exhibiting dominant inheritance with reduced penetrance. In these families severity of disease ranged from 15 to 113 degrees. Because there is no single model to adequately describe the inheritance of IS, we are applying both parametric and nonparametric analyses to the final datasets with the LINKAGE AND GENEHUNTER (GH) packages, respectively. When results were analyzed with GH some evidence for linkage ($p < 0.05$) at loci on chromosomes 5p, 6p, 10q, 16q, and 18q was obtained. Additional polymorphic loci are being genotyped in the three families to confirm and refine disease gene locations. All candidate loci are also being genotyped in 12 additional kindreds. Once linkage is confirmed by these combined analyses, positional candidate genes will be screened for mutations in affected members of linked families. This approach should identify novel genes for IS, and provide insights into the etiology of the disease.

Support for a role of HOXA1 in autistic spectrum disorders. *S.C. Robitaille¹, H. Zhang¹, J. Leggo¹, F. Macciardi², J.J.A. Holden¹.* 1) Queen's University and Ongwanada, Kingston, Ontario, Canada; 2) Centre for Addiction and Mental Health, Toronto, Ontario, Canada.

Autism spectrum disorders (ASDs) are a group of neurodevelopmental disorders affecting 1-2/1000 individuals and characterized by self-absorption, difficulties in verbal and non-verbal communication, a desire for sameness and repetitive behaviors. Early developmental anomalies of the external ears in thalidomide victims with autism have pointed to a prenatal insult between the 20th and 24th day of conception. HOXA1 has been suggested as a candidate gene for ASD since similar ear abnormalities are seen in Hoxa1 knockout mice (1). HOXA1 is one of the homeobox genes responsible for the anterior-posterior segmentation of the vertebrate hindbrain into rhombomeres and is therefore, involved in morphogenesis. Recently, a variant allele of HOXA1 was identified which is more frequent among individuals with ASD than their family members and unrelated individuals who do not have the disorder (2). We therefore tested whether two microsatellite markers (D7S2252 and D7S460) closely linked to HOXA1 showed linkage to or association with ASD in families with two affected children using the affected sib-pair and TDT methods.

Concordance was not significant (Maternal: N=74, $c^2=1.34$, $p=0.247$; Paternal N=81, $c^2=0.006$, $p=0.397$) but TDT results showed that a particular allele at D7S2252 was more frequently transmitted to affected offspring. These results suggest that a D7S2252 allele may be in linkage disequilibrium with a variation in the HOXA1 gene or another closely linked gene that contributes to susceptibility to ASD. Thus, additional studies on HOXA1 as a candidate gene are warranted; sequencing of the second exon is in progress. 1. Rodier PM *et al* 1996 Journal of Comparative Neurology 370:247-261 2. Stodgell CJ *et al* 1999 American Journal of Human Genetics 65:A371.

The Reln gene as a candidate locus for autism spectrum disorders. *H. ZHANG¹, C. Zhang¹, S. Robitaille¹, D.R. Grayson², A.R. Guidotti², F. Macciardi³, J. Leggo¹, J.J.A. Holden¹.* 1) Queen's University and Oshwanada, Kingston, On, Canada; 2) University of Illinois, Chicago, IL; 3) Center for Addiction and Mental Health, Toronto, ON, Canada.

Autistic disorder, the most severe of a group of pervasive developmental disorders (PDDs), is characterized by impairments in reciprocal social interaction, difficulties with both verbal and non-verbal communication, and displays of stereotypic activities. Genomic scans have implicated a region in the long arm of chromosome 7 as harboring a locus important in autism. The human Reln gene, mapped to 7q22, codes for a large extracellular protein secreted by Cajal-Retzius cells located in the marginal zone of the developing brain. It affects the signaling pathway through an interaction with an adapter protein (Dab1) expressed by the migrating cortical and hippocampal neurons and cerebellar Purkinje cells. Since Reln expression is significantly reduced (50%) in postmortem brains of patients with schizophrenia and bipolar disorder with psychosis, it is a reasonable candidate gene for autism spectrum disorder (ASD). We have recently identified a polymorphic trinucleotide repeat (CGG) in the 5' untranslated region (5' UTR) of the Reln gene exon 1. We tested whether there are expansions of this repeat or linkage to the Reln gene in 111 multiplex families with two or more siblings diagnosed with ASD (229 affected children and 215 available parents), and in 76 simplex families (76 affected children and 139 available parents). The number of CGG repeats varied from 6 to 18, with no expansion or contraction observed. Although there was no evidence of excess allele sharing in siblings (Mothers N = 43, $c^2 = 0.012$, $df = 1$, $p = 0.914$; Fathers N = 41, $c^2 = 0.012$, $df = 1$, $p = 0.912$), of interest the transmission disequilibrium test (TDT) showed that a particular allele was more frequently transmitted in a subset of the families studied, indicating a potential role for Reln in a subgroup of ASD. We are currently testing a highly polymorphic linked microsatellite locus (D7S618) to increase the number of informative families.

Apparent high prevalence of mitochondrial DNA mutations in congenital/ childhood non-syndromic sensorineural hearing loss. *T.P. Hutchin¹, K.R. Thompson¹, M. Parker³, V. Newton⁴, M. Bitner-Glindzicz⁵, R.F. Mueller².* 1) Molecular Medicine Unit, University of Leeds, St James's University Hospital, Leeds, UK; 2) Dept of Clinical Genetics, St James's University Hospital, Leeds, UK; 3) Dept of Clinical Genetics, Leicester Royal Infirmary, Leicester, UK; 4) CAEDSP, University of Manchester, Manchester, UK; 5) Institute of Child Health, University College London Medical School, London, UK.

Genetic factors are the major causes of childhood hearing impairment. Whereas autosomal recessive mutations account for the majority of prelingual non-syndromic sensorineural hearing impairment (NSSHI), the relative contribution of mitochondrial DNA (mtDNA) mutations to childhood onset NSSHI has not been established.

We screened for known mtDNA mutations associated with NSSHI in 202 randomly ascertained cases of congenital/ childhood onset NSSHI- 110 sporadic, 75 sib pairs and 17 families with affected individuals in more than one generation.

Four individuals (2%) were found to have mtDNA mutations: the 7472insC mutation was present in one sporadic case (0.9%) and the A7445G, T7510C and A3243G mutations were found in three families with affected individuals in more than one generation (17.6%). The latter all occurred in 3 of 10 (30%) of families where the hearing impairment was transmitted through the mother.

mtDNA mutations are thus likely to be a significant cause of childhood NSSHI, especially with a family history of hearing impairment which is transmitted through the mother. Routine screening for mtDNA mutations should therefore be considered in all cases of childhood hearing impairment, especially if the pattern of inheritance is consistent with maternal transmission.

Detection of mutations and heteroplasmy in the mitochondrial genome using denaturing high performance liquid chromatography. *P.E. McAndrew¹, J.M. Devaney¹, E.L. Pettit¹, E.I. Schwartz¹, D.A. Stephan², T.W. Prior³, M.A. Marino¹.* 1) Transgenomic Inc, Gaithersburg, MD; 2) Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC; 3) Department of Pathology, The Ohio State University, Columbus, OH.

The high mutation rate of mtDNA is due to the combined effects of the reactive oxygen species generated during oxidative phosphorylation, the lack of protective histones, and the lack of an efficient DNA repair mechanism. Defects in the human mitochondrial genome have been reported for several degenerative diseases, cancer, and aging. The normal sequence coexists with the mtDNA mutation for many degenerative diseases, and the proportion and distribution of this heteroplasmy often determines the severity of clinical symptoms. Several mtDNA mutations have been identified in cancer cell lines and primary tumors, and it has been suggested that mtDNA hypervariable regions represent somatic mutation hot spots which may be useful for cancer detection. An efficient method to rapidly screen the mtDNA regions implicated in human diseases and to scan the mitochondrial genome for somatic mutations in tumor samples would be a powerful tool for molecular diagnostic testing. We are currently using denaturing high performance liquid chromatography (DHPLC) as a method to detect mutations and heteroplasmy in mtDNA. DHPLC uses an ion-pair reversed-phase column to separate heteroduplex molecules from homoduplex fragments. We designed a multiplex assay and determined the optimal elution temperature and gradient conditions for several regions of the mtDNA genome, including the hypervariable regions. These conditions are being applied to evaluate several common mtDNA diseases including LHON, MERFF, and MELAS, and can be used to scan the mtDNA genome for somatic mutations in tumor samples. An SRM developed by NIST was used to evaluate heteroplasmy. We were able to detect as little as 1% heteroplasmy in a constructed sample with a silent mutation at position 6371 within the coding region. DHPLC is a rapid, versatile technology which will enable us to detect mutations in mtDNA and determine the degree of heteroplasmy in clinical samples.

Validity of the analysis of the FMRP expression in bloodsmears as a screening method for the Fragile X

Syndrom. *F.J. Ramos*¹, *M. Mila*², *M. Ortilles*¹, *M. Rife*², *B. Tazon*², *Y. De Diego*³, *R. Willemsen*³. 1) Secc. Genetica, Dpto. Pediatria, Facultad de Medicina, Universidad de Zaragoza, Zaragoza, Spain; 2) Serv. Genetica, Hospital Clinic, Barcelona, Spain; 3) Dept. Clinical Genetics, Erasmus University, Rotterdam, The Netherlands.

Fragile X Syndrome(FXS) is the leading cause of inherited mental retardation, affecting 1/4.000 males in the general population. Characteristic phenotypic findings include long face, large ears, machroorchidism and conective tissue anomalies. Its diagnosis is currently based on the presence of an abnormal CGG repeat expansion at the first exon of the FXS gene (FMR1) which causes the shutdown of transcription and subsequent absence of the FXS protein (FMRP). Currently, detection of patients and carriers of the FXS is routinely done by DNA analysis of the CGG repeat. In 1995, an FMRP antibody test was developed to detect the presence of FMRP in lymphocytes from bloodsmears. Subsequently, a pilot study in The Netherlands proved that, among males, the FMRP test was an useful screening method for the FXS. Here, we tested the validity of the FMRP test to screen males for Fragile X syndrome(FXS)in Spain. We studied 70 males with mental retardation (MR) of unknown etiology. Besides, 15 males with confirmed FXS and 100 normal males were also studied as controls. All the patients were clinically evaluated (including Hagerman's check-list and IQ) and tested for the FMR1 mutation (Southern and PCR). The FMRP test detected 2 patients (brothers) with the full mutation, whereas normal protein expression (x=86%, range 64-100%) was found in males with no abnormal expansion of the CGG in FMR1, as well as in controls (x=91%, range 70-100%). Males with FXS had an average FMRP expression of 11% (range 0-21%). These results support that the analysis of the FMRP expression in bloodsmears is a valid and simple method to screen males with MR of unknown etiology to rule out the FXS.

A novel mutation in mitochondrial DNA ATPase 6 gene is associated with progressive metabolic neurodegenerative disorder. *L. Li¹, P. Jayakar²*. 1) Dept.of Pediatrics, Childrens Hospital Los Angeles, Los Angeles, CA; 2) Clinical Genetics, Miami Childrens Hospital, Miami, FL 33155.

Progressive metabolic neurodegenerative disorders are associated with various nuclear and mitochondrial DNA mutations. During a routine mitochondrial DNA mutation screening, we found a novel mutation in mitochondrial adenosine triphosphate synthetase (mtATPase) gene from an 8-year old patient who was diagnosed with progressive metabolic neurodegenerative disorder. The mutation was confirmed as G-to-A transition at the nucleotide 8872 by dotblot and DNA sequencing, and the mutation created a new Hind III restriction endonuclease digestion site. We performed quantitative analysis on blood, muscle tissue, and cultured fibroblasts and the mutant genome were 10%, 9%, and 13%, respectively. The 8872 G-to-A mutation is mapped to the subunit 6 of ATP synthetase (ATPase 6), which is one of the complex V members. It causes amino acid changes from Glycine to Serine at a highly conserved position. The same mutation was not detected in the blood specimens from either mother or sister. At least four mutations in ATPase 6 have been identified for two related mitochondrial disorders: neurogenic atrophy, ataxia, retinitis pigmentosa (NARP) and maternally inherited Leigh syndrome.

Epigenetic differences in monozygotic twins and inbred animals. A. Petronis¹, V.S. Basile¹, I.I. Gottesman², A.D. Paterson¹, J.L. Kennedy¹, F. Macciardi¹, J. Nobrega¹, V. Pependikyte¹. 1) Neurogenetics, Centre for Addiction and Mental Health, Toronto, Ontario, Canada; 2) Department of Psychology, Gilmer Hall, University of Virginia, Charlottesville, VA.

Phenotypic discordance of monozygotic twins (MZ) is one of the mysterious issues in complex non-Mendelian diseases. Several genetic mechanisms, such as somatic mutations and skewed X inactivation in female twins, have been suggested but these are not common and therefore of limited explanatory value. New opportunities for investigation of the molecular substrate of differences in MZ twins arise if emphasis is shifted from DNA sequence-based factors to epigenetic DNA modification which regulates gene expression. In order to illustrate this idea, we applied the bisulphite modification technique for mapping of methylated cytosines in the regulatory region of the human dopamine D2 receptor gene (DRD2) in lymphocyte DNA from six pairs of monozygotic twins. In addition, we investigated methylation in drd2 promoter region of brain tissues from 12 inbred Sprague-Dawley rats, eight of them were treated with antipsychotic medication, haloperidol. The positions of methylated cytosines exhibited three types of variation i) within an individual (somatic mosaicism of epigenetic signals); ii) within the sets of MZ twins and among inbred animals; iii) across individuals belonging to different sets of MZ twins. For evaluation of epigenetic differences/similarities in the MZ twins two criteria were applied: 1) density of methylated cytosines, and 2) position of methylated cytosines. Three out of six MZ pairs exhibited a higher intra-pair similarity than any other randomly selected pair of unrelated individuals. In the epigenetic studies of animals, it was detected that haloperidol changed drd2 methylation status in the rats ($p < 0.04$), and this finding may serve as an example of environmental effects on epigenetic regulation of a gene. Epigenetic studies may lead to a better understanding of molecular mechanisms of phenotypic differences in genetically identical (or very similar) organisms.

A novel mice with respiration defects by introducing deleted mtDNA into zygotes. *Y. Goto*^{1,3}, *K. Nakada*^{1,2}, *K. Inoue*^{2,4}, *A. Ogura*⁴, *K. Isobe*², *I. Nonaka*^{1,3}, *J. Hayashi*^{2,3}. 1) National Inst. of Neuroscience, NCNP, Kodaira, Tokyo, Japan; 2) Inst. of Biological Sciences, Univ. of Tsukuba, Ibaraki, Japan; 3) Center for Tsukuba Advanced Research Alliance (TARA), Univ. of Tsukuba, Ibaraki, Japan; 4) National Inst. of Infectious Diseases, Tokyo, Japan.

Mice possessing pathogenic mutant mitochondrial DNA (mtDNA) would provide an ideal system to study how the mutant mtDNAs are transmitted through cell divisions and distributed in tissues resulting in expression of mitochondrial dysfunction and phenotype of mitochondrial diseases. To this end, we first made the cybrids by isolating the mouse mtDNA-less cells and trapping the deleted mtDNA which was spontaneously generated and accumulated in the brain of the aged mice. We then introduced the respiration-deficient cybrids into fertilized eggs and the adult mice were obtained. The mutant mtDNA was unexpectedly transmitted through generations and we found that the mutant mtDNA in the Fo-F3 individuals contained the duplication. The mutant mtDNA was distributed in the widespread tissues and cytochrome c oxidase deficiency was histochemically and/or ultrastructurally detected in several tissues examined including skeletal muscle and heart. The findings were quite similar to those seen in the human disease. Surprisingly the mice with high mutant content showed severe renal failure resulting in unexpected death. We further analyse the mice in more detail to understand the nature of mutant mtDNA origin, amplification and transmission through generations, and the pathophysiology of the human mitochondrial disease. The mice we generated can provide a powerful tool for the reserach on mitochondrial disease.

Quantification of mitochondrial DNA by real time Lightcycler PCR in Alzheimer's disease. *B. Rodriguez-Santiago¹, J. Casademont², V. Nunes¹*. 1) Medical and Molecular Genetics Center-IRO, Hosp.Llobregat, Barcelona, Spain; 2) Muscle Research Group, Hospital Clínic, IDIBAPS, Universitat de Barcelona, BCN, Spain.

Disturbances in mitochondrial DNA (mtDNA) and mitochondrial metabolism have been suggested to contribute to Alzheimer's disease (AD). MtDNA depletion leads to abnormalities in mitochondrial function. We have studied whether mtDNA depletion is a feature in the brain regions of 12 AD patients and 9 controls. Analysis in blood samples from 16 AD patients and 11 controls were also performed. Real time PCR based on Lightcycler (Roche Molecular Biochemicals, Germany) technology was used to determine the relative abundance of mtDNA in AD patients and controls. Results were normalized with nuclear ribosomal 18S (r18S) gene quantification. We found different amounts of mtDNA in the different brain regions both in AD patients and controls, but we did not observe mtDNA depletion in AD patients. We conclude that mtDNA depletion probably does not play a role in AD etiology. This work was supported by grants Fundació La Marató de TV3 2002/97 and 2102/97.

Novel cellular models of mitochondrial neurological disease. *A. Wong¹, L. Cavelier¹, H. Collins², M.F. Seldin², M.L. Savontaus³, M. McGrogan⁴, G.A. Cortopassi¹.* 1) Department of Molecular Biosciences, UC Davis, Davis, CA; 2) Row Program in Genetics, UC Davis, Davis, CA; 3) Department of Medical Genetics, University of Turku, Finland; 4) Layton Biosciences, Gilroy, CA.

Mitochondrial genetic disease frequently affects neural cells, however the pathophysiological mechanisms of disease have been mainly studied in non-neuronal cells, most frequently osteosarcoma cell lines. In an attempt to generate a more relevant cellular model system for the study of the neuropathophysiological mechanisms in mitochondrial genetic disease, we have demonstrated the transfer of mutant mtDNAs (mtDNA) from patients' lymphoblasts to a pre-neuronal cell line N tera2 (Nt2). Restriction digests were consistent with transfer of patient mtDNA, and homoplasmic lines were identified. A potential issue was the contamination of transmitochondrial cell lines with patient nuclear DNA, but assay of >50 variable microsatellite loci was inconsistent with nuclear contamination by donor cells. In addition, mtDNA and nuclear DNA copy number were similar in control and transmitochondrial cell lines. Nt2 cells bearing mutant mitochondria were differentiable with retinoic acid into postmitotic cells with a neuronal morphology. Such cells could represent a useful model in which to study the neuropathogenetic mechanism(s) of mtDNA mutations, and potential therapy.

Identification and analysis of cardiac mRNAs as targets for CUG triplet repeat binding protein. *D. Napierala, L. Timchenko.* Cardiovascular Sciences, Baylor College of Medicine, Houston, TX.

Myotonic dystrophy (DM) is a muscular disorder associated with abnormalities in many tissues including the heart. DM is caused by a CTG triplet repeat expansion in the 3' untranslated region of myotonin protein kinase (DMPK) gene. It is thought that expanded repeats in DMPK mRNA disrupt function of CUG-binding proteins, affecting expression of other CTG containing genes. In order to identify cardiac RNA targets for CUG-binding proteins, we screened the human heart cDNA library with CTG/CAG probe and identified a number of genes bearing perfect and interrupted repeats. Some of these repeats are polymorphic. Identified genes include those with well-characterized functions such as natriuretic factor type B (BNF) and myocyte enhancer factor (MEF2A) and genes with unknown functions. BNF and MEF2A mRNAs were selected for further study. BNF, a cardiac hormone with important vasoactive properties, contains an interrupted CAG repeat upstream of AUG initiation codon. MEF2A, a transcription activator regulating muscle differentiation and proliferation, contains a perfect CAG repeat within the open reading frame. To investigate whether CAG repeats within MEF2A and BNF mRNAs could be a binding site for CUGBP1, we analyzed interaction of CUGBP1 with RNA CAG repeats. CUGBP1 consists of three RNA-binding domains (RBD)s. Our studies showed that RBD1 and 2 bind only to CUG repeats, while RBD3 binds to both CUG and CAG repeats. The ability of RBD3 to interact with CAG repeats suggests that CUGBP1 might regulate BNF and MEF2A mRNAs via RBD3. It has been previously demonstrated that cytoplasmic CUGBP1 is associated with polysomes and is involved in regulating translation. To investigate the effect of CUGBP1 on translation of MEF2A, we used in vitro translation systems. We prepared the protein fractions purified from HeLa cells by ion exchange chromatography. Addition of fractions containing CUGBP1 and/or CUGBP1 immunoreactive proteins into a cell translation system programmed by MEF2A mRNA inhibited translation of MEF2A. These results suggest that CUG triplet repeat binding proteins could participate in regulating translation of cardiac CAG/CUG-containing mRNAs.

Overexpression of CUG repeats causes alterations in protein levels and activity of CUG triplet repeat binding protein, CUGBP1. *L.T. Timchenko¹, Z.J. Cai¹, T. Ashizawa², N.A. Timchenko³*. 1) Cardiovascular Sciences; 2) Neurology Department; 3) Pathology, Huffington Center on Aging, Baylor College of Medicine, Houston, TX 77030.

Myotonic Dystrophy (DM) phenotype is caused by expansion of CTG/CUG repeats in the 3' untranslated region of myotonin protein kinase (DMPK) gene/mRNA. Studies of DM pathogenesis indicated that expansion of CTG/CUG repeats affects gene expression on multiple levels including DNA replication, transcription, and RNA processing. We showed that a CUG triplet repeat RNA-binding protein, CUGBP1, is affected in DM patients, leading to alteration in splicing and translation of CUG containing RNAs. Here we present evidence that 1) overexpression of CUG repeats increases half-life of CUGBP1 and 2) major fraction of endogenous CUGBP1 in DM tissues is bound to RNA transcripts containing CUG repeats. In vitro studies showed that bacterially expressed purified CUGBP1 binds to RNA proportionally to the number of CUG repeat within RNA transcripts. To further investigate molecular pathways of CUG repeat dependent alterations of CUGBP1, we overexpressed RNA CUG repeats in cultured cells and measured the direct effect of CUG repeats on CUGBP1 protein. Overexpression of CUG repeats in cultured cells results in an increase of half-life of CUGBP1 and elevation of CUGBP1 protein levels. Half-life of CUGBP1 in HT1080 control cells is 3 hours; however, CUGBP1 levels are not reduced within 4 hours after blocking protein synthesis in cells expressing RNA CUG repeats. Similar to cultured cells, CUGBP1 is also induced in DM tissues. We developed a new HPLC based approach for the separation and analysis of CUGBP1-RNA complexes and free CUGBP1. This method was applied for studies of CUGBP1 in DM tissues. Analysis of CUGBP1-RNA complexes in DM heart showed that the majority of CUGBP1 is associated with RNA containing CUG repeats; however, in control samples, the majority of CUGBP1 is observed in RNA free fractions. These results are consistent with the hypothesis that increased number of CUG repeats in DM tissues leads to the elevation of CUGBP1 via titration and stabilization of the protein.

SCREENING OF CFTR AND PRSS1 MUTATIONS IN PATIENTS WITH PANCREATITIS. *A.L. Bernardino¹, D. Guarita², C. Mott², M.C.C. Machado², A.A. Laudanna², C.M. Tani², K.F. Miranda¹, F.L. Almeida¹, M. Zatz¹.* 1) Center for the Study of Human Genome, Universidade de S.Paulo, S.Paulo, Brazil Dept Biol, Univ de Sao Paulo, Sao Paulo, S.Paulo, Brazil; 2) Dept. Gastroenterologia, FMUSP, Univ. S.Paulo.

Mutations in the Cystic Fibrosis Transmembrane Conductance (CFTR) gene may be a risk factor for chronic pancreatitis. In addition, mutations in the Cationic Tripsinogen (PRSS1) gene cause hereditary chronic pancreatitis. We studied 73 unrelated patients with chronic pancreatitis (alcohol-related disease in 56, hyperparathyroidism in 1, idiopathic disease in 14 and hereditary disease in 2) and 4 patients with idiopathic acute pancreatitis. Screening of mutations in the CFTR and PRSS1 genes was performed through SSCP analysis and sequencing of abnormally migrating fragments. The PRSS1 gene in patients with hereditary pancreatitis was directly sequenced. The frequency of CFTR variants of T Tract length of intron 8 (IVS8Tn) was also investigated. No mutation was found in the PRSS1 gene. However 7 patients had mutations in the CFTR gene: 3 compound heterozygotes (delta F508/R851L, deltaF508/R170C, deltaF508/L206W) and 4 on just one allele (2 N/deltaF508, 1 N/P205S and 1 N/V920M). Seven mutations were found in the group of 14 patients with chronic idiopathic pancreatitis (25%) and 3 in the group of alcohol-related (2.7%). The 5T allele was found in 5.3% of the patients with alcohol-related chronic pancreatitis (which does not differ from the control population). However it was not seen in the idiopathic cases, in opposition to previous studies who found a relatively high frequency of this alleles (9.3%) in this group of patients. Interestingly the compound heterozygote (delta F508/R170C) male patient identified in the alcoholic group has two children. His only clinical symptom is pancreatitis suggesting that his mutation is associated with normal fertility and no pulmonary involvement. In addition our results suggest: a) the existence of another gene for hereditary chronic pancreatitis; b) in accordance to previous studies that mutations in the CF gene should be investigated in all patients with idiopathic chronic pancreatitis.

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Novel mutations and the emergence of a common mutation in the *SDHD* gene causing familial Paraganglioma.

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Familial paragangliomas (PGL) are slow-growing, highly vascular, generally benign neoplasms usually of the head and neck that arise from neural crest cells. This rare autosomal dominant disorder is highly penetrant and influenced by genomic imprinting through paternal transmission. Timely detection of these tumors may afford the affected individual the opportunity to avoid the potential serious morbidity associated with surgical removal and the mortality that may accompany local and distant metastases. Linkage to two distinct chromosomal loci, 11q13.1 and 11q23 has been previously reported. Recently, germline mutations in *SDHD*, a mitochondrial complex II gene on chromosome 11q23 have been demonstrated. We evaluated members of seven families with PGL, five previously studied and shown to have linkage to chromosome 11q23. The entire coding region of the *SDHD* gene was sequenced and yielded four novel mutations and one mutation shared in three of our unrelated families. Novel mutations found included a truncating mutation in exon 2, as well as a missense mutation, a deletion and an insertion in exon 4. Three of our families had a common mutation in exon 3 (P81L) that has been reported and thought to be a founder mutation. A restriction enzyme assay was developed for initial screening of this mutation. Molecular analysis is now available and recommended for presymptomatic diagnosis in those at-risk individuals and for confirmatory diagnosis in those having PGL. *SDHD*

Studies of HNF-1a mutations in Norwegian MODY3 families. *P.R. Njolstad^{1,2}, L. Bjorkhaug^{2,3}, L. Grevle³, H. Ye², Y. Horikawa², A. Molven³, O. Sovik¹*. 1) Dept Pediatrics, Haukeland Univ Hosp, Bergen, Norway; 2) Howard Hughes Medical Institute, Univ of Chicago, Chicago, Ill, USA; 3) Center for Medical Genetics and Molecular Medicine, Haukeland Univ Hosp, Bergen, Norway.

Background. MODY or Maturity-onset diabetes of the young is characterized by autosomal dominant inheritance, and onset usually before 25 years of age. Heterozygous mutations in the genes encoding glucokinase (GCK), and the transcription factors hepatocyte nuclear factor (HNF)-1a, 1b and 4a, and insulin promoter factor-1 can cause MODY by affecting pancreatic b-cell function.

Objective. To evaluate the type and prevalence of MODY gene mutations in Norwegian families with a clinical MODY diagnosis, and to investigate the molecular mechanisms responsible for the various phenotypic features observed for different mutations in the same genes.

Patients and methods. 24 Norwegian families with a clinical MODY phenotype were investigated. In addition to clinical and biochemical data, MODY genes were sequenced and some of the mutated genes analyzed with respect to DNA binding, transactivation activity and subcellular localization.

Results. Mutations in MODY genes were identified in 17 families. Two families had mutations in GCK. 14 families carried seven different mutations in HNF-1a; R131W, R171X, R263C and P291fsinsC in addition to the novel mutations G47E, P112L and Q466X. Also, one and two families had mutations in HNF-1b and GCK, respectively. Functional analysis of HNF-1a mutations indicated they were loss-of-function mutations with reduced DNA binding capacity (EMSA analysis) or impaired transcriptional activation (transactivation assay). Three mutations affected the nuclear localization of HNF-1a as revealed by immunolocalization.

Conclusions. HNF-1a diabetes is probably most prevalent in Norway. The dysfunction seems to be caused by reduced DNA-binding or transcriptional activity.

Screening of peroxisomal ABC genes in X-ALD patients. *M.B. Lachtermacher¹, K.D. Smith², B. Gerrard¹, M. Dean¹.* 1) LGD, National Cancer Institute, Frederick, MD; 2) Kennedy Krieger Institute, Baltimore, MD.

X-linked Adrenoleukodystrophy (X-ALD) is a neurodegenerative disease characterized by reduced peroxisomal very long chain fatty acid (VLCFA) β -oxidation in virtually all tissues. The X-ALD gene (ABCD1) belongs to the superfamily of ABC transporter genes. More than 300 different mutations have been described in this gene. The clinical expression of the disease shows a marked phenotypic variability where the most three common phenotypes are Cerebral Childhood ALD, Adrenomyeloneuropathy (AMN) and Addison's Disease. Extensive mutation analysis has demonstrated that there is no correlation between phenotype and genotype and different phenotypes can be found in the same family, suggesting that a modifier gene may play a role in determining the phenotype. The X-ALD protein shares high homology with other ABC peroxisomal proteins, named ALDR (ABCD2), PMP70 (ABCD3) and P70R (ABCD4). No diseases have been associated with these 3 genes and in vitro studies have shown that homo- as well heterodimerization occur between ABCD1, ABCD2 and ABCD3. To test the hypothesis that these ABC genes could play a role in determining the X-ALD phenotype, we performed association analysis based on the screening of the coding sequence as well as the promoter region of these genes in X-ALD patients presenting different phenotypes. The initial screening was carried out using the DHPLC technology followed by sequencing. We found 3 frequent Single Nucleotide Polymorphisms (SNP) that were present in more than 30% of the samples analyzed. One of the SNPs is located in the promoter region of the ABCD4 gene and the other two are located in the promoter and 3'UTR region of ABCD2. Our initial results show that there is no significant association between these variants and the final X-ALD phenotype.

Antipsychotic adverse reactions in schizophrenia : A pharmacogenetic study of weight gain and tardive

dyskinesia. *V.S. Basile¹, M. Masellis¹, V. Ozdemir¹, M.L. Walker¹, S.M. Quiterio¹, H.Y. Meltzer¹, J.A. Lieberman¹, S.G. Potkin¹, D. Mancama², M.J. Arranz², R.W. Kerwin², F.M. Macciardi¹, J.L. Kennedy¹.* 1) Neurogenetics Section, CAMH, University Toronto, Toronto, Ontario, Canada; 2) Dept. Psychological Medicine, King's College, University of London.

A drawback to treatment of schizophrenia is the occurrence of medication side-effects. Typical antipsychotics are known to induce movement disorders such as tardive dyskinesia (TD). Although atypical antipsychotics have a lower incidence of motor side-effects, their use is hindered by others such as weight gain. There is considerable variation among patients regarding their propensity to develop side-effects. It is likely that this variability is determined by a combination of genetic and environmental factors. We investigated the role of genetic factors in patient risk to develop typical antipsychotic induced TD and clozapine-induced weight gain. 112 typical antipsychotic treatment resistant schizophrenia patients were assessed for TD severity using the AIMS upon entry into a structured clozapine trial. During clozapine treatment, weight was assessed at baseline, 6 weeks and 6 months. Using ANCOVA, we found that the dopamine D3 receptor gene was associated with TD ($F[2,95]=8.25$, $p=0.0005$). The cytochrome P450 1A2 gene also revealed a significant association with TD ($F[2,99]=11.37$, $p=0.0007$). No significant associations were uncovered between 5HT2C genotypes and change in weight following 6 weeks ($F[2,75]=0.63$, $p=0.54$) and 6 months ($F[2,42]=0.13$, $p=0.88$) of clozapine treatment. Preliminary analyses have not shown associations between histamine H1 genotype and change in weight following 6 weeks ($F[2,71]=0.60$, $p=0.55$) and 6 months ($F[2,39]=0.04$, $p=0.95$) of clozapine treatment. Further analyses in larger samples are warranted and these samples are currently being collected. Although replication is necessary, this study supports a role for both DRD3 and CYP1A2 in the pathogenesis of TD. Psychiatric pharmacogenetics may in future lead to individualized pharmacotherapy based on the specific genetic, environmental and demographic characteristics of the patient.

Association of Tryptophan Hydroxylase (TPH) Gene with Smoking Behavior. *C. Lerman*¹, *N. Caporaso*², *A. Bush*¹, *L. Zheng*¹, *J. Audrain*¹, *P. Shields*¹. 1) Cancer Control Research, LCC, Georgetown Univ Medical Ctr, Washington, DC; 2) Division of Cancer Epidemiology and Genetics, National Cancer Institute Rockville, MD.

Approximately 50% of the variance in smoking behavior is attributable to genetic factors. Genes in the serotonin system are plausible candidates because of serotonin's role in mood regulation. The present study examined the association of smoking behavior with a polymorphism in the TPH gene, which codes for a rate limiting enzyme in the biosynthesis of serotonin. A polymorphism in intron 7 has been linked with a variety of traits involving poor impulse control. Participants in this study were 249 Caucasian smokers and 202 nonsmokers recruited through newspaper advertisements. Smokers completed smoking history and nicotine dependence assessments. The overall frequencies of the U and L allele were 42% and 58% , respectively. There was no association of TPH alleles with smoking status. However, case series analysis indicated that individuals with the U/U genotype started smoking at age 15.6 years, compared with 17.3 years among smokers with other genotypes. This association was significant in a multivariate regression model controlling for age, education, BMI, and alcohol use ($p=.01$). This finding is consistent with previous studies relating the U allele to impulsive behavior, and suggests that it may predispose to early smoking initiation. Future family-based studies are needed to confirm this finding.

Human Phenol Sulfotransferase1A1*2 Genetic Polymorphism in Type 2 Diabetic Patients. *M.M. Shi, M. Sinz, K. Rose, S.P. Myrand, M.R. Bleavins, F.A. de la Iglesia.* Departments of Worldwide Preclinical Safety, Pharmacokinetics, Dynamics, and Metabolism, Parke-Davis Pharmaceutical Research, Warner-Lambert Company, Ann Arbor, Michigan 48105.

Phenol sulfotransferase1A1 (SULT1A1) is a major enzyme isoform that catalyzes the sulfation of both small planar phenols and phenolic monoamines. A guanine to adenine substitution in the SULT1A1 gene leads to an amino acid change from arginine to histidine at codon 213 (SULT1A1*2). The objective of the current study was to compare the genotype and phenotype of this polymorphism. A total of 60 patients with type 2 diabetes were genotyped for SULT1A1*2 polymorphism using a TaqMan Allelic Discrimination assay. SULT activities were measured from peripheral platelets based on the sulfate conjugation of substrate 4-nitrophenol in the presence of [35S]-3-phosphoadenosine-5-phosphosulfate. Genotypic frequencies of the homozygous wild type, heterozygous and homozygous variants were 45%, 42% and 13%, respectively. Homozygous variant individuals have significantly lower enzyme activities (1.36 ± 0.28 nmol/mg/hour, n=8) than homozygous wild type individuals (3.96 ± 0.62 , n=27). Heterozygous individuals also have about a 30% decrease in enzyme activity (2.77 ± 0.33 , n=25) in comparison with homozygous wild type individuals, although it was not statistically significant. In conclusion, SULT1A1*2 polymorphism is associated with decreased enzyme activity in type 2 diabetic patients.

Isolation and analysis of Autism candidate genes on 7q. *P. Agbuya¹, A. Ashley-Koch¹, C.M. Wolpert¹, M.M. Menold¹, N. Matsumoto², S. Basu¹, D.M. Greenblatt¹, C.M. Powell³, M.L. Cuccaro⁴, D.H. Ledbetter², E.D. Green⁵, J.M. Vance¹, M.A. Pericak-Vance¹, J.R. Gilbert¹.* 1) Department of Medicine, Duke University Medical Center, Durham, NC; 2) Department of Human Genetics, University of Chicago, Chicago, IL; 3) Department of Pediatrics, University of North Carolina, Chapel Hill, NC; 4) WS Hall Psychiatric Institute, University of South Carolina, Columbia, SC; 5) NHGRI, National Institute of Health, Bethesda, MD.

Autistic Disorder (AD) is a progressive developmental disorder characterized by stereotyped patterns of behavior and deficits in language and social communication. Multiple AD total genomic screens have indicated the presence of an autism susceptibility locus within the distal arm of human chromosome 7 (7q31-q35). We have identified single-nucleotide polymorphisms in candidate genes (i.e. UBE2H, MEST/PEG, CPA2) within the candidate region for testing in our AD data set. In addition, we have identified a family, Duke Family 7543, in which AD affection status in males segregates with a paracentric inversion, Inv(7)(q22q31.2), located within the area of linkage. Utilizing 19 BACs for FISH and 11 probes for pulse field analysis, we have localized the distal breakpoint of this inversion to the most proximal 300 kb of NIH YAC contig O. The availability of genomic sequence has allowed us to utilize web-based exon prediction software (GENSCAN, GRAIL, FGENE) to search for novel genes in the breakpoint region. BLAST analysis of 20 predicted exons, followed by primer development and PCR amplification of fetal and adult brain cDNA libraries, have yielded the discovery of three novel transcripts. Extension and Northern analysis, respectively, are being used to determine transcript size and expression patterns of these transcripts, aiding in the determination of their status as autism susceptibility candidates.

CpG Island and Gene Mapping in the Austistic Disorder Region on Chromosome 15q11-13. *S.J. Kim, M.M. Menold, L. Zaeem, A. Ashley-Koch, M.P. Bass, J.M. Vance, M.A. Pericak-Vance, J.R. Gilbert.* Center for Human Genetics, Duke University Medical Center, Durham, NC.

Chromosome 15q11-q13 has been implicated in the genetic etiology of Autistic Disorder (AD). To isolate putative 15q11-q13 AD genes, a genomic contig and physical map of the 1-1.5 Mb region from the GABRB3 receptor to the OCA2 gene was generated. *Not* I and numerous *Eag* I restriction enzyme sites, frequently found in CpG islands associated with promoter and initiation sites at the 5' end of genes, were identified in this region.

To identify AD candidate genes within the contig, BAC and PAC clones spanning the region which contain *Not* I and *Eag* I sites were analyzed using Island Rescue PCR and other methods to isolate CpG islands. Sixteen sites, ranging in size form 0.5 to 3.5 kb, that have the characteristics of CpG islands, have been cloned and sequenced. Sequence data has allowed the mapping of a previously unmapped clone, which shows homology to the Mus T-cell receptor alpha-chain mRNA, and is expressed in brain, to the region. Putative coding sequences from CpG islands are being tested for the presence of SNPs to be used for association studies in our AD family data set. The isolation and fine mapping of these putative genes to the genomic contig and the indentificationa of additional potential CpG islands is ongoing. These studies will identify and map AD candidate genes and allow the investigation of the methylation status of CpG islands within the AD region in tissues.

Screening of autism candidate disease genes. *T.H. Wassink*¹, *J. Piven*², *V.C. Sheffield*³. 1) Department of Psychiatry, Univ Iowa College of Medicine, Iowa City, IA; 2) TEACCH, Univ of North Carolina College of Medicine, Chapel Hill, NC; 3) Department of Pediatrics and the Howard Hughes Medical Institute, Univ of Iowa College of Medicine, Iowa City, IA.

Efforts to find autism disease genes have identified a number of chromosomal regions of interest, including areas on 7q, 15q11-q13, and 13q. We, therefore, have begun to examine candidate disease genes in these regions in a sample of 72 families with at least two autistic siblings and 45 autism trios that were collected as part of the on-going Collaborative Linkage Study of Autism (CLSA). Genes were chosen for examination based on their physical location, function, expression pattern, and the amount of information available through public genetic databases. Genes were examined using a variety of techniques, including searching for and genotyping SNPs and STRPs from non-coding regions, as well as delineation and subsequent screening of exons for mutations. Polymorphisms were analyzed statistically for both linkage disequilibrium (LD) and allele sharing. Genes either partially or completely examined in this way to date include, from chromosome 7: *MEST*, *GRM8*, *UBE2H*, and *WNT2*; from chromosome 15: *HERC2*; and from chromosome 13: *UCHL3*. Thus far, only one SNP in the 3'UTR of *WNT2* has shown suggestive evidence for LD ($TDT\ c^2 = 2.845$, $p = 0.09$), and no disease causing mutations have been identified.

Unique RET genotypes comprising specific haplotypes of polymorphic variants predispose to isolated

Hirschsprung disease. *S. Borrego*^{1,2}, *A. Ruiz*², *M.E. Saez*², *O. Gimm*¹, *X. Gao*¹, *M. Lopez-Alonso*², *A. Hernandez*², *F.A. Wright*¹, *G. Antinolo*², *C. Eng*¹. 1) Human Cancer Genetics, Ohio State University, Columbus, OH; 2) Hospital Universitario Virgen del Rocío, Seville, Spain.

Hirschsprung disease (HSCR) presents with functional intestinal obstruction due to aganglionosis of the gut. Germline mutations of the RET protooncogene is said to cause 50% of familial and 30% of isolated cases. Population-based studies indicate RET mutations account for 3% of isolated HSCR. To determine if specific haplotypes of RET polymorphisms predispose to HSCR, we looked for association of RET haplotype(s) and HSCR in cases vs region-matched controls. 7 loci across RET were typed and haplotypes formed for HSCR, their unaffected parents and the controls. Haplotype and genotype frequencies and distributions were compared among these groups using transmission disequilibrium test and standard case-control statistic. 12 unique haplotypes (A-L) comprising combinations of alleles at the 7 loci, were obtained. The distributions of haplotypes between cases and controls ($p < 0.0001$) and cases and non-transmitted parental haplotypes were different ($p < 0.0001$). Genotypes comprising pairs of haplotypes were formed for cases and controls. There were 38 different genotypes among cases and controls combined. Genotype distribution between cases and controls was distinct ($p < 0.0001$). For example, BB, BC, BD and CD, all of which contain at least one allele with the polymorphic A45A, are prominently represented among HSCR, together accounting for >35% of the case genotypes, yet these 4 genotypes are not represented in the controls. Conversely, AA, AG, DD, GG and GJ, none of which contain A45A, together account for 43% of the control genotypes, yet, they are never seen among HSCR. Our data suggest that genotypes comprising specific pairs of RET haplotypes are associated with predisposition to HSCR either in a simple autosomal recessive manner or in an additive, dose-dependent fashion. This novel predisposition, whether the polymorphisms per se or another locus in linkage disequilibrium likely represents a common low penetrance predisposition for the majority of all isolated cases of HSCR.

A Functional Difference in the Promoter Region of the NRAMP Gene between Patients with Ulcerative Colitis and Crohn's Disease. *M.R. Eichenberger, V. Young, L.E. Hunt, S. Galandiuk.* Department of Surgery, University of Louisville, Louisville, KY.

Ulcerative Colitis and Crohn's disease collectively known as inflammatory bowel diseases are autoimmune diseases that affect approximately one million Americans. In up to 40% of these patients it is difficult to accurately differentiate between these two entities. The natural resistance associated macrophage protein gene (NRAMP1) is located on chromosome 2q35 and encodes a transmembrane protein with uncertain function. This gene has been implicated in the susceptibility to both tuberculosis and autoimmune diseases. In a previous study, a known (GT)_n polymorphism in the promoter region of NRAMP1 dramatically affected promoter efficiency. We therefore investigated whether this polymorphism was informative in respect to diagnosis of inflammatory bowel disease. Genomic DNA from patients with ulcerative colitis (57), Crohn's disease (91) and unrelated controls without inflammatory bowel disease (19) was extracted and genotyped with the use of PCR and autoradiography. Subjects were matched for age and ethnicity. The frequencies of two genotypes were statistically different between disease groups. The genotype 2,3 was elevated in Crohn's disease 40% (36/91) relative to ulcerative colitis 19% (11/57) ($\chi^2=5.23$, $p=0.022$). Whereas, the genotype 3,3 was elevated in ulcerative colitis 70% (40/57) relative to Crohn's disease 47% (43/91) ($\chi^2=6.575$, $p=0.01$). These results suggest an association between the specific types of inflammatory bowel disease and the promoter region of the NRAMP1 gene.

Schizophrenia and IL-1, MTHFR and HLA DQB1 markers among the chinese. *K.V Chowdari¹, K. Xu², F. Zhang¹, C. Ma², T. Li², B.Y. Xie², J. Wood¹, D.E. Downard¹, M. Trucco³, N. Saha⁴, W.A. Rudert³, V.L. Nimgaonkar¹.* 1) Department of Psychiatry, WPIC, Pittsburgh, PA. E-mail chowdarikv@msx.upmc.edu; 2) Guangzhou Psychiatric Hospital, Guangzhou, China; 3) Departments of Pediatrics and Human Genetics, University of Pittsburgh, Pittsburgh, PA 15213, USA; 4) Department of Pediatrics, National University of Singapore, Singapore.

Using cases and unrelated controls, we examined putative associations between schizophrenia and three candidate gene polymorphisms. Two independent cohorts were available: 1. Guangzhou, China: patients with schizophrenia (DSM IV criteria, n=130) and controls (n=130); 2. Singapore: patients with schizophrenia (ICD 10 criteria, n=140) and controls (n=120). HLA DQB1 (ch 6p21.3) To follow up our reported associations in the Singapore cohort, the Guangzhou cohort was genotyped at HLA DQB1 and 5 flanking markers. Significant case-control differences were noted at HLA DQB1 and DQCAR, a short tandem repeat marker approximately 1 kb telomeric to HLA DQB1, supporting an association in the HLA Class II region. Interleukin-1 gene cluster (IL-1, 2q13-q21) Three polymorphisms were investigated: bi-allelic markers at IL1A (-889) and IL1B (-511) and variable number of tandem repeats (VNTR) at IL1RN. Haplotypes were also estimated using a likelihood based method. No significant case-control differences were detected with respect to genotypes, haplotypes or allele frequencies in either cohort. Methylene tetrahydrofolate reductase gene (MTHFR, 1p36.3) A C to T substitution at position 677 was investigated. Significant case-control genotype differences were present in the Guangzhou cohort, but not in the Singapore cohort. No significant allele frequency differences or deviations from Hardy Weinberg equilibrium were present.

An HphI polymorphism in exon 2 of the E-selectin gene as a genetic marker of premature coronary artery disease. *F. Zheng, S.Q Ye, D. Virgil, L.Q Zhang, P.O Kwiterovich.* Pediatrics, Johns Hopkins University, Baltimore, MD.

An increased expression of E-selectin has been observed on arterial endothelium interacting with leukocytes and macrophages in human atherosclerotic lesions. We examined whether a polymorphism in the E-selectin gene, due to a G to T mutation (98G>T) in exon 2 untranslated region, predicted premature coronary artery disease(CAD). Other lipid and nonlipid risk factors including another polymorphism in the E-selectin gene, a substitution of arginine for serine (S128R), were also examined. By restriction mapping, we found that G98>T mutation abolished the HphI site. We designed a new method with the following primers (Forward, 5'-TGCCCAAATCTTAGGATGC-3'; Backward, 5'-AAGCCCAGGGAAGAACACAT-3') to amplify a 332-bp fragment of the E-selectin gene using the polymerase chain reaction. When the HphI recognition site was present, this fragment was digested into 194 and 138 fragments. We determined three genotypes (GG, GT, TT) of the E-selectin gene in 101 patients aged ≥ 45 years for men and ≥ 55 years for women who underwent elective diagnostic coronary arteriography at the Johns Hopkins Hospital (51 patients had CAD, 50 did not have CAD). The frequency of the T-allele mutation was significantly higher in patients with CAD than in controls (0.22 vs. 0.10, $p=0.024$). Multiple logistic analysis of significant predictors of CAD (Table 1) showed that both the G98>T and S128R mutations in the E-selectin gene were significant predictors of premature CAD. These mutations may affect E-selectin gene expression and promoting atherogenesis. Table 1. Multiple logistic analysis of the significantly different CAD risk factors. The parameters are listed in the order: Variables, Odds Ratio (95%, CI) and P value. 1).98G>T, 3.58 (1.20-10.67), $P=0.022$; 2) S128R, 4.11 (1.24-13.56), $p=0.020$; 3) Total Cholesterol, 0.99 (0.97-1.07), $p=NS$; 4) Triglyceride, 1.00 (0.99-1.01), $p=NS$; 5) LDLapoB, 1.02 (0.99-1.05), $p=0.071$; 6) Smoking, 5.87 (1.84-18.75), $p=0.003$.

SCA8 CTG expansion: evidence for a common haplotype and highly mutable region on both ataxia and non-ataxia chromosomes. *M. Moseley, J. Jacobsen, C. Liquori, A. Durand, L. Davis, Y. Ikeda, T. Bird, R. Spiegel, T. Ashizawa, M. Pandolfo, N. Potter, F. Schaefer, A. Milunsky, J. Kennedy, W. Seltzer, L. Atwood, J. Vincent, J. Day, L. Ranum.* Inst Hum Genet, Minneapolis, MN.

We recently reported that an untranslated CTG expansion causes spinocerebellar ataxia type 8 (SCA8). To investigate the possibility that a separate mutation in linkage disequilibrium with a founder chromosome predisposed to CTG expansion could account for nonpathogenic expansions observed in the general population, we performed haplotype analysis on expansion chromosomes from 24 ataxia families, 14 SCA8 samples from Athena Diagnostics, 14 families with psychiatric diseases, and 7 controls. Although initial studies with markers 100-200 kb from the CTG repeat did not detect a common haplotype, subsequent analysis with 8 flanking STR markers we developed (at 72, 56, 52, 24, 9, 13, 17 and 20 kb) detected a highly conserved haplotype among the ataxia and psychiatric patients and controls. These results indicate that a separate mutation closely linked to the repeat is unlikely to account for the reduced penetrance of SCA8. TDT testing of haplotypes established using 5 of the markers further supports an ancestral origin of the expansion ($p=0.0001$). Distinct haplotypes were only observed among 4 Japanese ataxia families and two Athena samples. Although the majority of CTG expansions arose from a common founder, substantial length variation of the adjacent (CTA)₃₋₁₇ repeat and mutations within the CTG tract are observed among and within families. In addition, we have identified an SNP 90 bp 3' of the CTG tract that is observed in only 3 ataxia families with the common haplotype. Although this SNP is unlikely to play a role in ataxia, it suggests that the region of high mutability extends beyond the CTA/CTG repeat. To examine the evolutionary conservation of the SCA8 repeat, genomic DNA from gorilla and chimpanzee was examined. Surprisingly, both have significant (CTA)_n tracts ($n=16$ and 19 , respectively) but each has only a single CTG. Although we have previously demonstrated that CTG length plays an important role in penetrance, other sequence changes within or near the repeat may also affect pathogenesis.

Mouse tissue culture models of unstable triplet repeats: *in vitro* selection for larger alleles, mutational expansion bias and tissue specificity, but no association with cell division rates. *M. Gomes-Pereira, M.T. Fortune, D.G. Monckton.* Division of Molecular Genetics, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK.

Simple tandem repeats are found throughout mammalian genomes and often show high levels of length polymorphism. In particular, the expansion of CAG•CTG trinucleotide repeats has been associated with several human diseases. Once into the expanded disease associated range, repeat instability becomes dramatic, not only in the germline, but also throughout the soma. This gross instability is expansion biased and contributes toward the unusual genetics, tissue specificity and progressive nature of these disorders. Such expansions describe a unique form of dynamic mutation, whose mechanism is still poorly understood. It is generally assumed that repeat length changes arise via replication slippage, yet no direct evidence exists to support this hypothesis in a mammalian system. We have previously generated transgenic mouse models of unstable CAG•CTG repeats that replicate the dynamic nature of somatic mosaicism. We have now used tissues from these mice to establish cell cultures. Monitoring of repeat stability over time in these cells has revealed the progressive accumulation of larger alleles. Surprisingly, cells carrying longer repeats are selected during the first few passages of the cultures, quickly giving rise to a much larger average repeat size than observed in the progenitor tissue. However, the establishment of single cell clones has confirmed the further accumulation of mutations *in vitro*. The highest levels of instability are observed in cultured kidney cells, whilst the transgene remains very stable in lung cells. Eye cells exhibit intermediate stability. These tissue-specific dynamics parallel the previous *in vivo* observations. No correlation between repeat stability and the cell proliferation rate has been found, rejecting a simple association between length change mutations and cell division and implying a role for additional cell-specific factors. The development of this mouse cell model provides an exciting new system to study the molecular biology of trinucleotide sequences.

The antisense/sense transcriptional organization of SCA8/KLHL1 is conserved in mouse. *K.A. Benzow, M.D. Koob.* Inst Hum Genet, Univ Minnesota, Mpls, MN.

Spinocerebellar ataxia type 8 (SCA8) is caused by a CTG expansion in the untranslated SCA8 transcript, an endogenous antisense RNA that overlaps the Kelch-like 1 (KLHL1) gene. The KLHL1 promoter and open-reading frame are conserved in mouse, and we now report that a KLHL1-antisense transcript is present in mouse as well. The evolutionary conservation of this antisense/sense transcriptional organization strongly indicates that KLHL1-antisense transcripts such as SCA8 play a significant biological role in both man and mouse, presumably as a regulator of KLHL1 expression.

SCA8 RNA and the mouse KLHL1-antisense RNA are transcribed from homologous promoter regions in the first intron of KLHL1 and extend through the transcription and translation start sites as well as the first splice donor sequence of KLHL1. The mouse antisense RNA is not spliced and terminates in a polyadenylation site in the KLHL1 promoter region, whereas the SCA8 RNA is variably spliced into processed transcripts that contain up to 5 exons and terminate at one of two alternative polyadenylation sequences 23 or 31 kb 5' of KLHL1. The mouse KLHL1-antisense transcript was detected in RNA isolated from the cerebellum and from total adult brain and total fetal tissue, but was not detected in RNA isolated from other adult tissues except, perhaps significantly, in testes and ovary. Similarly, SCA8 RNA is present in various brain tissues, including the cerebellum, the tissue most affected by SCA8, but was not detected in the other adult tissues tested to date. By characterizing human and mouse KLHL1-antisense RNA both *in situ* and in derived tissue culture systems we hope to determine how the KLHL1 sense and antisense transcripts interact and what role these interactions may play in the pathology of SCA8.

Evidence for a stepwise expansion of the CGG repeats within the FMR1 gene. *S.-H. Chen^{1,2}, B. Xu^{1,2}, J.M. Shoof², N.E. Buroker¹, C.R. Scott^{1,2}.* 1) Department of Pediatrics, University of Washington, Seattle, WA; 2) Children's Hospital & Regional Medical Center, Seattle, WA.

Fragile-X syndrome is a common form of inherited mental retardation. It is caused by an expansion of CGG repeats within the FMR1 gene. The number of repeats is variable with 6-54 repeats being considered normal; 52-200 repeats being unstable and labeled as "premutations," and >200 repeats being associated with the fragile-X syndrome. A further division of CGG repeat sizes consists of those in the range of 41-60 and labeled as being an "intermediate," but typically stable. We have determined the CGG repeat sizes in Asian and Caucasian populations in Seattle. The Caucasians had a higher percentage (2.5%) of the intermediate alleles than did those in the Asian population (~1%). Three polymorphic markers (DXS548/FRAXCA1/INV10+14C/T) were analyzed for their association with FMR1 CGG repeats in each of the populations. There were significant haplotype differences between normal, fragile-X and intermediate chromosomes in each population. In Caucasians, the haplotypes 20-18-C/T, 21-18-C/T and 25-21-C/T were associated with the fragile-X and intermediate chromosomes, while 20-19-C/T was associated with the normal chromosomes. In Asians, only 21-18-C/T was associated with the fragile X and intermediate chromosomes, while 20-18-C/T and 20-19-C/T were associated with normal chromosomes. Since specific haplotypes are associated with intermediate (41-60 repeats) and fragile-X alleles in both Caucasian and Asian populations, it implies that the intermediate alleles are precursors of a stepwise expansion to premutation alleles and then to fragile X alleles.

Molecular chaperones affect aggregation, toxicity and solubility of expanded polyglutamine repeat androgen receptor in a cellular model of SBMA. *C.K. Bailey^{1,2}, D.E. Merry²*. 1) Univ of Pennsylvania, Neuroscience Graduate Group, Philadelphia, PA; 2) Thomas Jefferson Univ, Dept. of Biochemistry and Molecular Pharmacology, Philadelphia, PA.

Neuronal inclusions are found in many neurodegenerative diseases, including the family of polyglutamine repeat (polyQ) diseases, indicating that protein misfolding is a common feature. PolyQ diseases, including Spinal and Bulbar Muscular Atrophy (SBMA), are characterized by ubiquitinated intranuclear inclusions, which are associated with the molecular chaperones, Hsp40 and Hsp70, and with components of the 26S proteasome. This suggests that misfolded expanded polyQ-containing proteins are bound by molecular chaperones in an unsuccessful refolding attempt, and then targeted to the proteasome for degradation.

To study the role of Hsp40 and Hsp70 in the pathogenesis of SBMA, we are using a cell culture model in which Cos-7 and neuronal MN-1 cells are transiently transfected with androgen receptor (AR), the gene which is mutated in SBMA. We showed previously that expression of expanded repeat, truncated AR (ARexp), but not normal repeat AR, resulted in aggregate formation and cellular toxicity. Co-expression of HDJ-2/Hsdj, an Hsp40 family member, with ARexp decreases AR aggregation. Interestingly, a J domain mutant is also able to decrease aggregation, suggesting that HDJ-2 may function independently of its Hsp70 interaction to reduce aggregation. Toxicity assays reveal that HDJ-2 also decreases the toxicity associated with ARexp.

In order to understand how these chaperones affect ARexp aggregation and toxicity, we examined their effect on AR solubility. Overexpression of HDJ-2 does not result in a substantial increase in soluble AR, suggesting that its effects on aggregation and toxicity are mediated by another mechanism. On the other hand, Hsp70 significantly increases AR solubility, and HDJ-2 enhances this effect. Our continued studies on chaperones should help to elucidate the roles of these proteins on polyQ disease pathogenesis and may lead to therapeutic strategies for SBMA and other polyQ diseases.

Proteolytic processing of the truncated androgen receptor is associated with cell death. *I. Andriola¹, C.K. Bailey^{1,2}, D.E. Merry¹*. 1) Dept.Biochem, Molec Pharm, Thomas Jefferson Univ, Philadelphia, PA; 2) Univ of Pennsylvania, Neuroscience Graduate Group, Philadelphia, PA.

Spinal and bulbar muscular atrophy (SBMA) is one of an increasing number of human neurodegenerative disorders caused by a CAG/polyglutamine-repeat expansion. The observation that neuronal intranuclear inclusions in motor neurons of SBMA patients contain only N-terminal epitopes of the expanded repeat androgen receptor (AR) suggests that this mutant AR is proteolyzed during SBMA pathogenesis. Indeed, studies of Huntington's Disease and DRPLA also reveal that proteolytic processing may be involved in the pathogenic mechanism. We have developed a cell culture system in which expression of a truncated expanded repeat AR (N133) causes cellular toxicity coupled with aggregation and proteolytic processing. To study the role of proteolytic processing of the AR in cell death, neuronal MN-1 cells were transiently transfected with normal and expanded repeat forms of the N133 AR (17Q, 66Q, 98Q). Western blot analysis revealed the nearly complete restriction of a cleaved NH₂-terminal AR peptide to floating dead cells (confirmed by caspase-3 activation), while the full N133 protein was found in both viable adherent and floating cells. Inhibition of caspases with the inhibitors ZVAD-FMK and DVED-FMK had no effect on the fragment production still detectable in floating cells, indicating that cleavage was not due to one of the inhibited caspases or to caspase activation. To determine if proteolytic cleavage is associated with aggregation, we characterized Triton X-100 soluble- and insoluble-fractions with regard to fragment distribution. We found repeat length-dependent enrichment of the N-terminal fragment in a Triton X-100-insoluble fraction. Overexpression of the molecular chaperones Hdj-2/HSDJ or Hsp70 dramatically increased the solubility of this fragment. Together, these data indicate that the N-terminal fragment is particularly prone to misfolding and aggregation. In addition, the formation of this fragment is strongly correlated with cell death. Characterization of the cleavage site by mass spectrometry in order to further understand the role processing in cell death is underway.

Progressive neurological disease in mice expressing reduced amounts of huntingtin with 20 and 111 glutamine repeat. W. Auerbach^{1, 2}, M.S. Hurlbert², P. Hilditch-Maguire⁵, Y. Zaim Wadghiri², V.C. Wheeler⁵, S.I. Cohen⁵, A.L. Joyner^{1, 2, 4}, M.E. MacDonald⁵, D.H. Turnbull^{2, 3}. 1) Howard Hughes Medical Institute; 2) Skirball Institute Of Biomolecular Medicine; 3) Departments of Radiology and Pathology; 4) Departments of Cell Biology, Physiology and Neuroscience, New York University School of Medicine, New York, NY; 5) Molecular Neurogenetics Unit, Massachusetts General Hospital East Charlestown, MA.

Compound heterozygous mice in which the two copies of the Huntington's disease homolog gene (Hdh) have been altered to express reduced levels of huntingtin protein with 20 and 111 glutamine repeats do not show the embryonic lethal defects of Hdh null mutants, but nevertheless all surviving HdhneoQ20/neoQ111 mice develop a progressive neurological phenotype. All HdhneoQ20/neoQ111 mice are smaller than their wild type and heterozygous littermates from birth, but otherwise appear normal. Starting at about 8 weeks of age they develop a progressive movement disorder which includes hind limb claspings, difficulties in walking, and paralysis of the limbs and tail. At the most advanced stage, HdhneoQ20/neoQ111 mice become hypokinetic and die. Immunoblot analysis of brain protein extracts from HdhneoQ20/neoQ111 mice detected greatly reduced levels of huntingtin with both 20 and 111 glutamines. Magnetic resonance micro-imaging and neuropathological evaluation of brain sections showed enlarged lateral and third ventricles in about half of the HdhneoQ20/neoQ111 mice. However, the ventricle enlargement did not progress with age, and, no overt cell loss was observed, suggesting that the enlarged ventricles appears to result from developmental defects due to reduced embryonic huntingtin expression. As expected, with time some huntingtin protein was detected in the nucleus of striatal neurons. Taken together, our data indicate that a reduction in the level of huntingtin can result in developmental abnormalities, but that in all animals leads to a progressive neurological phenotype that may be associated with expression of the Q111 form of huntingtin in the adult brain.

Multiple founder effects in spinal and bulbar muscular atrophy (SBMA, Kennedy disease) around the world. A.

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Spinal and bulbar muscular atrophy (SBMA, Kennedy disease) is an X-chromosomal recessive late-onset neurodegenerative disorder caused by death of the spinal and bulbar motor neurons and dorsal root ganglia. The patients often show also signs of partial androgen insensitivity. SBMA is caused by a CAG repeat expansion in the first exon of the androgen receptor (AR) gene. Our earlier study suggested that all the Nordic patients with SBMA originated from an ancient Nordic founder mutation, but the new intragenic SNP marker ARd12 revealed that the Danish patients derive their disease chromosome from another ancestor. The Finnish, Swedish and Norwegian patients carry a same kind of haplotype with absent StuI site, while the patients from Denmark and from all the other countries studied harbour the StuI site. In search of relationships between patients from different countries, we haplotyped altogether 123 SBMA families from around the world for two intragenic markers and 16 microsatellites spanning 25 cM around the AR gene.

Two different founder haplotypes were seen among the German SBMA patients. The Italian patients showed three entirely different founder haplotypes, and the Japanese patients had two distinct haplotypes. All the Canadian patients carried different haplotypes, but two of them harboured similar haplotypes to the two different Australian founder haplotypes. The fact that different SBMA founder haplotypes were found in patients from around the world implies that the CAG repeat expansion mutations occur relatively often. No expansion prone haplotype could be detected.

Trinucleotide diseases often show correlation between the repeat length and severity and earlier onset of the disease. A negative correlation between the CAG repeat length and the age of onset was found in the 95 SBMA patients with defined ages at onset.

A Spinocerebellar Ataxia Type 1 pedigree in Taiwan: Clinical and Molecular Analysis. *M. Hsieh¹, T.M. Lew⁴, Y.Y. Chen², C.Y. Yang³, C.J. Tsai³, M.L. Peng⁵, S.Y. Li¹.* 1) Department of Life Sciences; 2) School of Medical Technology; 3) Institute of Medicine, Chung Shan Medical and Dental College; 4) Department of Neurology; 5) Department of Ophthalmology, Chung Shan Medical and Dental College Hospital, Taichung, Taiwan, Republic of China.

Spinocerebellar ataxia type 1 (SCA 1) is an autosomal dominant disorder characterized by neurodegeneration of the cerebellum, spinal cord and brainstem. This disease is associated with expansion of unstable CAG repeats within the coding region of the gene, which has been located on 6p22-p23. In this study, we have screened 81 indexed patients with dominant inherited ataxia disorders for the SCA1 mutation by radioactive genomic polymerase chain reaction (PCR). An SCA 1 pedigree was identified in Taiwan for the first time; ten members of the kindred including three patients, three at-risk individuals and four normal individuals underwent our genetic and neurological examinations. Cardinal clinical features including cerebellar ataxia, pyramidal and extrapyramidal signs, and ocular manifestations were detected in symptomatic patients; ocular dysmetria, palmomental sign, hyperreflexia and peripheral visual field impairment were observed in symptomatic patients and most of the at-risk individuals. In addition, we demonstrated that a non-radioactive method, consisting of the direct visualization through an ethidium bromide stained agarose gel, can be used to accurately screen individuals for SCA1 mutations in a timely fashion. In summary, we describe: i) the first identified SCA1 pedigree in Taiwan, ii) symptoms and signs of the SCA1 patients and premonitory signs of at-risk individuals, iii) visual field change of all members with CAG expanded SCA1 gene, and iv) a method of molecular analysis to quickly detect SCA1 mutation. Taken together with our previous results, SCA1, as well as SCA7, were much less frequent (1.2%, 1 family for each genotype) in our collection, compared to SCA3/MJD accounting for 29.6% of the cases (24 families), SCA2 accounting for 9% (9 families), and the remaining families were negative for SCA6 and DRPLA mutations.

FMR1 CGG expansion to full mutation: What is the lower limit in premutation females? *S.L. Nolin¹, F. Rousseau², G.E. Houck¹, A.D. Gargano¹, V. Biancalana³, L. Hinkle⁴, H. Hjalgrim⁵, E. Holinski-Feder⁶, F. Kooy⁷, J. Longshore⁸, J. MacPherson⁹, J. Mandel³, G. Matthijs¹⁰, S. Sherman⁴, P. Steinbach¹¹, M.L. Väisänen¹², H. von Koskull¹³, W.T. Brown¹*. 1) Institute for Basic Research, Staten Island, NY; 2) Université de Laval, Quebec, Canada; 3) Laboratoire de Diagnostic Génétique, Faculté de Médecine et CHRU Strasbourg, France; 4) Emory University, Atlanta, GA; 5) The John F. Kennedy Institute, Glostrup, Denmark; 6) LMU-München, München, Germany; 7) Dept of Medical Genetics, University of Antwerp, Antwerp, Belgium; 8) Greenwood Genetic Center, Greenwood, SC; 9) Wessex Regional Genetics Laboratory, Salisbury Wiltshire, UK; 10) Center for Human Genetics, Leuven, Belgium; 11) Universität Ulm, Ulm, Germany; 12) Oulu university Hospital, Oulu, Finland; 13) Helsinki University Central Hospital, Helsinki, Finland.

In fragile X premutation females, expansion to a full mutation is a function of CGG repeat length with larger repeats having a greater risk for expansion. For repeat sizes from 50-60 the risk of expansion to a full mutation is uncertain. We have carried out a collaborative study to identify the smallest premutation allele undergoing expansion to a full mutation in one transmission. Thirteen laboratories contributed DNA from their smallest premutation females with full mutation offspring. The FMR1 CGG repeats in these alleles were amplified by PCR in one laboratory and the products were analyzed in parallel. The smallest alleles were sized exactly by comparison to alleles of premutation males defined by sequence analysis. The smallest premutations that expanded to a full mutation in one transmission were alleles with 59 repeats. This allele size was observed in two samples, from two different laboratories. The two samples with 59 repeats were identified from more than 2000 premutation females among the 13 laboratories. These results suggest that full mutation expansions from 59 repeat alleles occur infrequently and that full mutation expansion from smaller alleles are unlikely.

Modulating huntingtin half-life alters polyglutamine-dependent aggregate formation and cell toxicity. *M.D. Kaytor, J.C. Iber, S.T. Warren.* Departments of Biochemistry, Genetics and Pediatrics, Emory University School of Medicine and Howard Hughes Medical Institute, Atlanta, GA.

Huntington disease is a neurodegenerative disorder caused by expansion of a glutamine-encoding CAG trinucleotide repeat within the *IT15* gene. This expansion results in a pathogenic stretch of glutamines in the gene product, huntingtin. The expanded CAG tract appears to be the source of toxicity, as longer tracts lead to an earlier age of onset and a more severe clinical phenotype. A common finding among the polyglutamine expansion disorders is intracellular protein aggregates, either in the nucleus or the cytoplasm. Although these aggregates do not initiate disease, the finding that they are generally ubiquitinated implicates the ubiquitin-proteasome pathway in neuronal degeneration. Furthermore, components of the 26S proteasome have been found in the aggregates and heat shock proteins are likely involved in their metabolism. This suggests that cells may be unable to properly dispose of them via the ubiquitin-proteasome pathway. To investigate the mechanism of formation of these aggregates, we developed a cell culture model to express huntingtin containing either a pathogenic or non-pathogenic polyglutamine tract engineered to have an altered half-life. Fusion proteins that contain the first 171 amino acids of huntingtin, fused to the enhanced green fluorescent protein (EGFP) were designed to alter the degradation rate through the ubiquitin-dependent N-end rule pathway, by varying the N-terminal amino acid. Huntingtin-EGFP with a pathogenic CAG tract and a shorter half-life displays a delayed onset of aggregate formation. Aggregates containing this type of huntingtin are less toxic to the cells than those containing huntingtin with a pathogenic CAG tract and a longer half-life. Huntingtin-EGFP with a non-pathogenic CAG tract and a long or short half-life produces few aggregates and minimal cell toxicity. These data suggest that rapid clearance through the ubiquitin-proteasome pathway slows aggregate formation and reduces cell toxicity, illuminating a potential route of therapeutic intervention.

Transcriptional regulation by huntingtin. *L. Jones¹, J. Duce¹, C. Hartog¹, L. Elliston¹, R. Caswell³, F. Wilkinson⁴, G.E. Morris⁴, Nguyen thi Man⁴, J. Neal², P.S. Harper¹.* 1) Inst Medical Genetics, Univ Wales Col Medicine, Cardiff, Wales, UK; 2) Dept of Pathology, Univ Wales Col Medicine, Cardiff, Wales, UK; 3) School of Biosciences, Cardiff University, Cathays Park, Cardiff, Wales, UK; 4) MRIC Biochemistry Group, NE Wales Institute, Plas Coch, Wrexham, LL1 2AW, Wales, UK.

Huntington's disease (HD) is an autosomal dominant neurodegeneration caused by an expanded polyglutamine tract in the huntingtin protein. We have characterised an interaction of huntingtin with the nuclear receptor co-repressor (N-CoR), narrowing the areas of interaction to interacting area II in the C-terminal region of N-CoR and the polyglutamine tract in huntingtin. N-CoR represses transcription from a number of sequence specific DNA binding receptors, including the thyroid hormone and retinoic acid receptors, using a histone deacetylase mediated mechanism. In vitro reporter assays indicate that as well as demonstrating an interaction with N-CoR, N-terminal huntingtin is capable of repressing transcription in a repeat-length dependent manner. We are investigating whether this repression is related to the interaction with N-CoR, and find that the repression is dependent on histone deacetylase activity. We have also used immunolocalisation to determine the co-localisation of huntingtin and N-CoR in cell culture and in human HD and control brain. In early stage HD brain, Vonsattel grades 0-II, we find huntingtin localised diffusely in the nucleus of some pyramidal neurons from cortical layers V and VI and in a proportion of striatal neurons; very few or no intranuclear inclusions are observed in these brains from adult onset patients. This diffuse nuclear staining was observed with antibodies generated to N-terminal and also more C-terminal regions of huntingtin. In cell culture co-transfection of huntingtin and N-CoR demonstrates differential co-localisation of these proteins depending on the polyglutamine repeat length of huntingtin. Our evidence is consistent with the involvement of N-CoR in mediating transcriptional repression by huntingtin.

Polymorphisms of the SOX1 Protein and the Risk of Neural Tube Defects. *J.S. Nye^{1,2}, J. Luo^{1,4}, S.G. Guy¹, E. Melvin³, D.G. McLone², J. Charrow², J.F. Sarwark², M.C. Speer³, NTD Collaborative Group³.* 1) Molecular Pharmacology & Pediatrics, Northwestern Univ. Med.School, Chicago, IL; 2) Children's Memorial Hospital, Chicago, IL; 3) Center for Human Genetics, Duke Univ. Med. Ctr., Durham, NC; 4) present address: Orthopedic Surgery, Univ. Chicago.

Neural tube defects (NTDs) are a frequent component of the 13q deletion syndrome. Recently we identified an individual with a small deletion of distal 13q33-34 and a myelomeningocele. This case and published 13q deletions indicated a critical haploinsufficiency region for NTDs encompassing 13q33-34 (Luo et al, Amer. J. Med. Genet. 91: 227, 2000). In order to identify genes from distal 13q that contribute to the risk of NTDs, we began a screening of genes residing in this region that have roles in neural development at the time of neurulation. Sox1, a member of the SRY family of transcription factors, is expressed in the developing neural tube and affects neurogenesis in cultured embryonal carcinoma cells. Its sequence shows perfect and imperfect GGC/GCG repeats encoding one polyglycine and four polyalanine domains. All five of these domains were polymorphic. Detailed analysis of the region encoding the fourth polyalanine repeat was performed in 504 controls, 462 NTD patients, and 361 of their parents. We observed an in-frame insertion allele (insGV) in controls, but found other in-frame insertions and deletions exclusively in NTD families. Additionally, individuals homozygous for the insGV allele were identified only in families with NTDs. These data suggest that SOX1 may contribute to the risk of NTDs.

Investigating the cause of macroorchidism in fragile X syndrome using oligonucleotide microarrays. *W.T. O'Donnell, S.T. Warren.* Biochemistry, Emory Univ. School of Medicine, Atlanta, GA.

Fragile X syndrome is an X-linked dominant mental retardation disorder caused by the absence of Fragile X Mental Retardation Protein (FMRP). Patients exhibit moderate mental retardation, subtle somatic signs, and, in post-pubescent males, macroorchidism. This later sign has been difficult to understand, as the testis histology from patients is largely normal. As in humans, *Fmr1* knockout mice exhibit macroorchidism. To gain insight into FMRP function and the consequences of its absence in the testis, we set out to identify genes whose expression levels differ between wildtype and *Fmr1* knockout mice. Towards this end we isolated RNA from the testes of 8-week-old wildtype and knockout littermates and probed oligonucleotide arrays. Of 19,000 genes and ESTs examined, 90 showed significant change in knockout compared to wildtype. Changes detected on the chips ranged from 2-fold with an average intensity change of 4000 for more abundant transcripts to over 20-fold with an average intensity change of 500 for less abundant transcripts. Those genes with the largest change were confirmed by quantitative fluorescent RT-PCR. Identification of the differentially expressed genes should prove insightful as to the cause of macroorchidism in fragile X syndrome and into the function of FMRP in general.

Janus effects of premutation size CGG repeats on gene expression. *G. Raca, E. Siyanova, S. Mirkin.* Dept Molecular Genetics, Univ Illinois, Chicago, IL.

Fragile X Syndrome is one of the most common forms of inherited mental retardation and the first disease described to be the result of expansion of unstable trinucleotide repeats. Patients with Fragile X have massive expansion (over 200 copies) of a polymorphic CGG repeat in the 5' untranslated region of the FMR1 gene, which causes a silencing of the FMR1 promoter. For a size range of a CGG tract between 50 and 200 copies, a term "premutation length" was introduced to describe the propensity for allele expansion in the absence of direct clinical symptoms. The second part of this definition is being seriously questioned lately, since both phenotypic changes as well as changes in FMR1 mRNA levels in premutation carriers have been described. This warrants a systematic investigation of the effects of premutation lengths of CGG repeats on gene activity. To conduct such a study, we cloned 35, 50 and 70 (CGG)_n(CCG)_n repeats in two different orientations into the 5'UTR of the luciferase gene and analyzed their influence on reporter gene activity upon transient transfection into the SW480 cells. To our surprise, tested repeats caused a significant increase in luciferase activity only when CCG was in the sense strand for transcription. RNase protection studies showed that (CGG)_n(CCG)_n repeats in both orientations led to an increase in the amount of luciferase mRNA. At the same time, in vitro translation experiments demonstrated that CGG but not CCG repeats reduce translation efficiency relative to the control. Taken together, these results suggest that the seemingly normal level of luciferase activity for the CGG constructs were obtained due to a counterbalance between an increased mRNA level and its decreased translation efficiency. Our findings indicate that premutation-length repeats, at least in some cell types, are not neutral with regard to gene activity, but can affect both transcription and translation. The opposing effects of CGG repeats on transcription and translation can be balanced in such a way that the final gene activity stays mostly unchanged. This can explain tolerance for premutation length expansions in humans and contribute to understanding the variability and inconsistency of symptoms in premutation carriers.

Meiotic stability of a fragile X premutation CGG trinucleotide repeat in a mouse model. *W.J. Paradee, S.T. Warren.* Departments of Biochemistry, Pediatrics and Genetics, Emory University School of Medicine and Howard Hughes Medical Institute, Atlanta, GA.

Fragile X syndrome is due to the unstable expansion of a CGG repeat within the FMR1 gene. The mechanism of this expansion, and of other trinucleotide repeats, is unknown. Mouse models fail to fully recapitulate the instability seen in humans and most often reveal little or no instability of transgenes derived from unstable human alleles. However, the precise interpretation of these studies is difficult as the transgenes tested are not X-linked nor are they transmitted in a diploid context. Since full expansion of the FMR1 premutation occurs almost exclusively from the human female, it is possible that meiotic interaction between the normal and premutation alleles is involved in the massive expansion. We developed a mouse model to specifically address this question. We homologously targeted the FMR1 repeat tract from alleles containing either 81 or 21 repeats to the identical genomic locus, immediately upstream of the X-linked Hprt locus. Like previous studies, transmission of either allele independently was stable. Heterozygous female mice, carrying one allele with 81 repeats and the other with 21 repeats, were mated to wildtype males to more fully mimic human transmission. To date, 34 offspring have been analyzed from 4 such breeding pairs without any evidence of expansions or contractions. Thus, we have most closely modeled the transmission of the FMR1 premutation in humans without evidence of instability. While further experiments are ongoing using these animals, for example to test if the parental origin of the premutation allele in the female is important, our data is consistent with the notion that mice have cellular mechanisms which stabilize the premutation repeat which may be absent or modified in humans.

Identification of candidate trans-acting genetic modifiers that bind to the vicinity of CAG repeat in HD. K.

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by specific neuronal loss and dysfunction in the striatum and cortex. HD is caused by an expansion of a CAG triplet repeat, that is translated into a polyglutamine tract, in exon 1 of the HD gene. However, the molecular mechanism of the CAG repeat expansion is still unknown. Studies on the HD transgenic mice and the HD families have demonstrated that the repeat instability was influenced not only by the number of CAG repeats but also by sex. These results suggest that the cis-acting sequences in conjunction with the association of the trans-acting factors play roles in repeat instability. To identify cis- and trans-acting genetic modifiers relating to the CAG repeat expansion, we explored the DNA binding proteins that bind to the 5'-region (-364~+158) of the human HD gene containing 20 CAG repeats. Using a yeast One-Hybrid System with the 5'-region of the human HD gene as a reporter gene, we screened the cDNA library from human testis and identified four positive clones (clone 2, clone 4, clone 8 and clone 11). DNA sequencing analysis of clones 2 and 8 revealed that two clones encoded for novel proteins. Analysis of the deduced amino acid sequences revealed that putative proteins encoded by them shared similar domain structures : a single zinc finger domain and the C-terminal conserved region (86% identity). By northern blot analysis, transcripts of clones 2 and 8 were found to be ubiquitously expressed and their sizes were 1.5kb and 5.5kb, respectively. These results suggest the presence of the cis-acting elements within the 5'-region of the human HD gene and their associated trans-acting factors. Further analyses of the positive clones including detection of the DNA binding activity, the DNA binding site in vitro and the screening of full length cDNA are currently being carried out.

Six5 loss results in lens, bone and muscle defects *Six5*. *P.S Sarkar, J. Han, S. Reddy.* Institute for Genetic Medicine, USC Keck School of Medicine, Los Angeles, CA 90033. email: psarkar@hsc.usc.edu.

CTG expansion in myotonic dystrophy (DM) results in decreased expression of SIX5, a homeo-domain encoding gene, located 3' of the repeat tract. To test the role of SIX5 gene in the etiology of DM we have developed a strain of mice in which the *Six5* gene was functionally inactivated. Loss of *Six5* results in lens, bone and muscle defects. *Six5*^{-/-} mice are sterile, demonstrating that *Six5* is required for normal gametogenesis and/or embryogenesis. *Six5* mutant animals show a 10-20% reduced body weight and approximately 10% of *Six5*^{+/-} and *Six5*^{-/-} mice demonstrate muscle wasting at 2-3 months of age. Muscle of the jaw, neck and diaphragm were found to be severely affected. Consistent with the loss of these muscle, we observe secondary defects of the bone, including curvature of the spine, mandibular dislocation and over-eruption of the incisors. Molecular analysis of the *Six5*^{-/-} skeletal muscle demonstrates that *Six5* loss results in abnormal expression of putative *Six5* target genes, including myogenin and alpha 1 subunit of the Na/K pump. These data suggest that decreased expression of *Six5* may contribute to DM skeletal myopathy. *SIX5*.

Suppression of polyglutamine repeat cytotoxicity in *Drosophila*. *L.M. Thompson*¹, *A. Kazantsev*³, *H.A. Walker*², *D.E. Housman*³, *J.L. Marsh*². 1) Dept Biological Chemistry, Univ California, Irvine, Irvine, CA; 2) Dept Developmental and Cell Biology, University of California, Irvine; 3) Dept of Biology, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA.

Expanded repeats of polyglutamines are responsible for several human neurodegenerative diseases including Huntington disease. A hallmark feature of these diseases is the appearance of neuronal intranuclear inclusions preceding the onset of symptoms. We have designed a suppressor peptide to bind to and interfere with aggregation mediated by expanded repeat huntingtin protein in cell culture. The coding sequence for this suppressor peptide was inserted into a *Drosophila* expression vector and transgenic animals were produced. Suppressor expression alone was non-toxic and showed cytosolic localization. The peptide was then expressed in the same genetic background as an expanded polyglutamine (polyQ) repeat peptide. *Drosophila* that express the expanded polyglutamine repeat peptide alone show a dominant cytotoxicity, neuronal degeneration and the formation of neuronal intranuclear inclusions. When expanded polyglutamine repeat peptide is co-expressed with the suppressor peptide in neurons, photoreceptor neuronal degeneration is rescued and in at least one case, dominant lethality is suppressed. In addition, the suppressor peptide appears to be redistributed into neuronal intranuclear inclusions *in vivo* when co-expressed with the expanded polyQ peptide. These results support a novel approach to develop potential therapeutic agents for polyglutamine repeat diseases.

Nondysmorphic infant with mosaic complete paternal uniparental isodisomy and normal biparental inheritance.

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A growth retarded, eight month old girl with an ASD, hepatomegaly, enlarged left second toe, dysphagia, hypotonia and developmental delay, underwent bilateral adrenalectomy for nodular adrenal hyperplasia. A diagnosis of McCune-Albright syndrome (MAS) was considered, but evaluation of known mutations of the α -subunit of the stimulatory G-protein of adenylate cyclase (GNAS1) on 20q13.2 was negative. Her complex presentation, including MAS-like problems, raised the possibility of uniparental disomy (UPD) for chromosome (chr) 20. Short tandem repeat (STR) markers for chr 20 showed paternal isodisomy (PID). Unexpectedly, evaluation of other chr STRs revealed that the patient's blood DNA had complete PID. Complete PID was demonstrated at every STR on every chr tested in adrenal, urine sediment, and liver DNAs. Since PID for an entire haploid set causes complete hydatidiform molar pregnancy, we assumed that the patient was a chimera composed of a fused zygote with PID and a zygote with normal biparental inheritance (BPI). DNA from buccal cells showed BPI as expected. However, the paternal haplotype was the same as that observed in the PID clone. Thus, this infant is likely a mosaic arising from a single zygote that formed a cell line with complete PID and another cell line with normal BPI. Recently, Haaf et al.(AJHG 65:A18#89) showed in mice that parental genomes have topological separation for the first few cell divisions after fertilization. We believe this patient demonstrates that this phenomenon occurs in humans. Her unusual molecular situation can be explained by the formation of an abnormal isodisomic clone by reduplication of the paternal genome during the normal separation of parental chromosomes during early post fertilization cell division. Her complex phenotype is the combined result of paternally inherited mutant recessive genes and the loss of function of genes expressed in other tissues that require maternal imprinting. A limited distribution of complete tissue PID likely ameliorates the severity of the phenotype. This infant illustrates the importance of demonstrating normal BPI of other chromosomes when UPD of a single chromosome is detected.

Phenotypic analysis of Silver-Russell syndrome patients with maternal uniparental disomy for chromosome 7.

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Silver-Russell syndrome (SRS) is a syndrome of severe intrauterine and postnatal growth retardation with slight dysmorphic features including a triangular face with a bossing forehead, a small lower jaw, and down turned mouth corners, clinodactyly and brachydactyly of the fifth digits, and asymmetry of body, face and/or limbs. The etiology of SRS is still unclear but recently maternal uniparental disomy of chromosome 7 (matUPD7), the inheritance of both chromosomes from only the mother, has been found in approximately 10% of SRS patients. In a systematic screening for cases of maternal uniparental disomy (matUPD7) among SRS patients, we have identified four matUPD7 cases out of 32 SRS patients studied (12.5%). A thorough phenotypic evaluation of these SRS patients revealed that the four matUPD7 patients present with many common characteristics to each other and seem to form a phenotypically homogenous group. They consistently lack some classical features of SRS, such as a significantly triangular face, micrognathia, and down turned mouth corners. However, all present with additional specific features such as speech delay, severe feeding difficulties, and excessive sweating. These features are only seldom noted in non-matUPD7 SRS patients. It has been proposed that one or more imprinted genes influencing growth and development reside on human chromosome 7 and that they have a role in the etiology of SRS, especially in cases of matUPD7. The typical characteristics of SRS vary to a great extent among individuals and thus SRS patients form quite a heterogenous group. Thorough clinical evaluation of four matUPD7 SRS patients and comparison of their phenotypes to 28 non-matUPD7 SRS patients suggests that matUPD7 patients have a mild SRS phenotype and they may form a distinct phenotypic entity among SRS patients.

Genes, other than TLR4, are involved in the response to LPS. *E. Lorenz*¹, *M. Jones*², *C. Wohlford-Lenane*¹, *N. Meyer*¹, *K.L. Frees*¹, *D.A. Schwartz*¹. 1) Dept Pulmonary & Critical Care, Duke Univ Medical Ctr, Durham, NC; 2) Biostatistics, University Of Iowa, Iowa City.

To determine whether genes other than TLR4 were involved in the response to LPS, we genotyped 18 strains of mice for TLR4 and evaluated their response to inhaled LPS (airway hyperreactivity, lavage cellularity, and lavage cytokine concentration). Of the 18 strains tested, 6 strains were wild type for TLR4 and 12 strains had mutations in TLR4. In fact, of those strains with TLR4 mutations, 9 had mutations in highly conserved residues. Among strains wild type for TLR4, the airway response to inhaled LPS showed a phenotype from very sensitive, such as DBA/2, to hyporesponsive, such as C57BL/6. Strain C57BL/6 had 83.1 % (+/- 1.92) polymorphonuclear leukocytes (PMNs) in the lavage fluid and strain DBA/2 had 96.67% (+/- .67) PMNs. Differences between these strains were detected as well in the airway response to inhaled methacholine and the concentration of cytokines and chemokines in the lavage fluid following inhalation of LPS. Specifically, the levels for TNF- α , ICAM-1 and IL-6 were significantly higher in strain DBA compared to strain C57BL/6 following inhalation of LPS. Although the TLR4 mutant strains C3H/HeJ and C57/10ScCr were phenotypically distinct, the other strains with mutations for TLR4 demonstrated a broad distribution in the physiologic and biologic response to inhaled LPS. The results of our study demonstrate that while certain TLR4 mutations can be linked to a change in the LPS response phenotype, additional genes are clearly involved in determining the LPS response in mammals. Additional studies involving genes regulating LPS induced airway hyperreactivity and airway inflammation should yield a clearer picture of the other genes essential in the innate immune response in mammals.

The biological basis for autosomal dominant nonsyndromic hearing loss, *DFNA15*: expression, DNA binding and transcription properties. *K.B. Avraham*¹, *S.L. Khare*², *M. Xiang*², *S.J. Dawson*³, *S. Weiss*¹. 1) Department of Human Genetics & Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel; 2) Center for Advanced Biotechnology & Medicine & Department of Pediatrics, UMDNJ-Robert Wood Johnson Medical School, Piscataway, N.J. 08854 USA; 3) Department of Molecular Pathology, University College London Medical School, London W1P 6DB UK.

A mutation in the *POU4F3* gene is responsible for *DNFA15* (MIM 602459), autosomal dominant nonsyndromic hearing loss, found in an Israeli family, Family H. The 8 bp deletion (884del8) forms a frameshift and removes most of the homeodomain. Targeted deletion of both alleles of *Pou4f3* causes complete deafness in the mouse, but aged heterozygous knock-out mice show no sign of hearing impairment, suggesting that haploinsufficiency does not cause deafness in Family H. Alternatively, the Family H mutation might be acting in a dominant-negative fashion.

To gain insight into the mechanisms that lead a *POU4F3* mutation to cause progressive hearing loss in humans, we investigated its expression, DNA binding and transcription properties. We hardly observe any binding of the protein product of the 884del8 mutation to a *Pou4f* consensus DNA binding site by EMSA. Moreover, this mutated product does not appear to interfere with binding of *POU4F3* and *POU4F2* to the consensus site. *In vivo*, the truncated *POU4F3* was found to be translated and stable in transient transfections. Most dramatic, most of the truncated *POU4F3* protein does not enter the nucleus but remains in the cytoplasm. Using a luciferase reporter gene assay, we show that the truncated transcription factor is less active in regulating the *SNAP25* promoter than the normal one. We are currently examining the potential dominant-negative effect of the mutated protein. Another possible mechanism for the Family H mutation is that there is aggregation of misfolded mutated protein, which when reaching a certain threshold in the cell, leads the cells to death. The role of *POU4F3* in gene regulation and in Family H pathogenesis indicates that this protein is a major component in the auditory cascade.

Cloning of genes involved in non-syndromic hearing impairment. *M.W.J. Lijndijk¹, A.I. den Hollander¹, V. van Limpt¹, H.G. Brunner¹, H. Kremer², F.P.M. Cremers¹.* 1) Department of Human Genetics, University Medical Center Nijmegen, Nijmegen, The Netherlands; 2) Department of Otorhinolaryngology, university Medical Center Nijmegen, Nijmegen, The Netherlands.

In this project it was proposed to: i) isolate novel human cochlea-specific genes, ii) map them in the human genome, and iii) investigate 5-10 cochlear genes for their putative involvement in familial hearing impairment.

For this purpose a fetal cochlea cDNA library was created, which was enriched for cochlea specific cDNAs. This was done through a suppression subtractive hybridization (SSH) technique (Diatchenko et al., PNAS 93:6025-6030, 1996; Clontech PCR-Select cDNA Subtraction Kit). In this procedure, full-length cochlea cDNA was digested with a 4-basepair recognizing restriction enzyme, *RsaI*, subtracted with excess non-cochlear cDNA from several tissues, amplified exponentially and cloned into a plasmid vector.

Analysis of a first set of 128 clones revealed 54 new clones, 40 clones that match a known EST and 18 clones with homology to a known human gene. From the 128 clones 58 clones were selected for further analysis, based on their novelty or because their EST expression profile suggested a possible involvement in defects of the inner ear. By employing a semi quantitative RT-PCR an expression profile was determined for these clones. cDNAs were selected based on the following criteria: 1) unique expression in fetal cochlea, 2) expression in cochlea higher than all other tissues, 3) expression in fetal cochlea and a maximum of two other tissues. These cDNAs were localized in the human genome with a PCR based mapping procedure. Several of these cDNAs indeed mapped near loci involved in hearing impairment, for example DFNB7/11 (9q13-q21), DFNA30 (15q26), DFNB16 (15q21-q22) and DFN3 (Xq21.1). These clones will be studied in more detail by isolating the full length cDNA sequence, determining the intron-exon structure, and by mutation analysis in relevant families.

Novel mutations in the USH2A gene. *D.J. Orten, C.A. Carney, M.D. Weston, W.J. Kimberling.* Boys Town National Research Hospital, Omaha, NE.

Usher syndrome type II is an autosomal recessive disorder with moderate to severe hearing loss and progressive retinitis pigmentosa (RP). Mutations in the USH2A gene on human chromosome 1q41 cause approximately 70% of Usher II cases, affecting approximately 4000 people in the United States. This gene encodes Usherin, a protein with a predicted molecular weight of 171.5 kD that contains domains observed in extracellular matrix or cell adhesion proteins. There are four readily identifiable domains in Usherin: laminin type VI (LN), laminin epidermal growth factor (LE), fibronectin type-III (FN) and a unique amino terminal domain. The gene is encoded by 21 exons and spans 105 kb. Because the domains have distinct functions, it is reasonable to expect that the severity of the phenotype will depend not only on the type of mutation, but on which domain is disrupted. Previous mutation searches of Usher II probands have revealed 31 distinct mutations in 14 of the 21 exons, with 2299delG, a frameshift leading to a stop codon in exon 13, as the most frequent (16%). Although missense mutations were reported in all 4 domains, the majority occurred in the laminin type VI domain. One missense mutation (C759F), in the LE domain, was reported in 10 patients with RP, and normal hearing. Our purpose was to identify novel mutations and determine the distribution of mutations in Usherin. Usher IIa probands were screened for the common mutation by an amplification refractory mutation detection system (ARMS) assay. Heteroduplex and sequence analysis was then used to screen 93 independent Usher II cases who lacked the common mutation. Nine novel mutations were identified in the first 11 Usherin exons, including 4 new missense mutations in the laminin type VI domain. Additional screening of Usher IIa families for Usherin mutations will indicate amino acids within each domain that are important for Usherin function, and may identify additional mutations associated with phenotypic variation. Furthermore, identification of families that do not have mutations in Usherin is essential for reducing the impact of heterogeneity on the search for other Usher II genes. Supported by NIH-NIDCD/2 P01 DC01813-07 and the Foundation Fighting Blindness.

Deafness resulting from mutations in the GJB2 (connexin 26) gene in Brazilian patients. *E. Sartorato¹, C. Oliveira¹, F. Alexandrino¹, A. Guerra².* 1) CBMEG, Laboratorio de Genetica Humana, Universidade Estadual de Campinas, Campinas, SP, Brazil; 2) Depto. Genetica Medica, FCM, Universidade Estadual de Campinas, Campinas, SP, Brazil.

Congenital deafness occurs in approximately 1 in 1000 live births. In developed countries half of this hearing loss is genetic. However, in Brazil the majority of cases of hearing loss are due to environmental factors, such as congenital infections (mainly rubella), perinatal anoxia, kernicterus and meningitis. It has been demonstrated that connexin 26 (GJB2) gene is a major gene for congenital sensorial deafness. Mutations in this gene cause 10%-20% of all genetic sensorial hearing loss, becoming a watershed for research in this area. One specific mutation, 35delG (also referred to as 30delG), accounts for the majority of mutant alleles. Surprisingly, the extent of the hearing impairment vary from mild/moderate to profound, even within the homozygous group for the common 35delG mutation, also can be progressed with age. Mutation analysis in the GJB2 gene and audiology were performed on 32 families (42 patients) presenting at least one child with congenital hearing loss. Five different connexin 26 mutations were identified, including one novel mutation. Mutations in the GJB2 gene were found in 30% of the subjects tested. This finding should facilitate diagnosis of congenital deafness and allow early treatment of the affected subjects.

GJB2 mutations in Greek patients with prelingual deafness. A. Pampanos¹, M. Grigoriadou¹, T. Antoniadis², K. Grønskov³, K. Brøndum-Nielsen³, T. Iliadis⁴, N. Voyiatzis⁴, J. Economides⁵, P. Neou⁵, N. Apostolopoulos⁵, A. Skevas⁵, M.B. Petersen¹. 1) Dept Genetics, Inst Child Health, Athens, Greece; 2) Dept Genet & Mol Biol, MITERA Matern & Surg Center, Athens, Greece; 3) Dept Med Genet, JF Kennedy Inst, Glostrup, Denmark; 4) Aristotle Univ Thessaloniki, Thessaloniki, Greece; 5) Greek Deafness Consortium.

Mutations in the gene encoding the gap-junction protein connexin 26 (GJB2) on chromosome 13q11 have been shown as a major contributor to prelingual, sensorineural, non-syndromic, recessive deafness in Caucasian populations. One specific mutation, 35delG, has accounted for the majority of the mutations detected in the GJB2 gene and is one of the most frequent disease mutations identified so far with highest carrier frequency in Southern European populations. We have previously detected a carrier frequency of the 35delG mutation of 3.5% in the Greek population. In a collaboration with the major referral centers for childhood deafness in Greece, patients were examined by an extensive questionnaire to exclude syndromic forms and environmental causes of deafness and by allele-specific PCR for the detection of the 35delG mutation. The 35delG mutation was found in 32.9% of the alleles in 164 unrelated cases of prelingual deafness: 49 homozygotes and 10 heterozygotes. Individuals heterozygous for the 35delG mutation were further analysed by direct genomic sequencing of the coding region of the GJB2 gene, which revealed R184P and 486insT mutations in single alleles. We conclude that the 35delG GJB2 mutation is responsible for one third of prelingual, sensorineural deafness in Greece, which is higher than the usually quoted 20% for Caucasian populations.

DFNB28, a novel locus for prelingual nonsyndromic autosomal recessive hearing loss maps to 22q13 in a large consanguineous Palestinian kindred. *T.D. Walsh¹, H. Shahin^{2, 3}, J. Morrow¹, M-C. King¹, E. Lynch¹, K. Avraham², M. Kanaan³.* 1) Medical Genetics, University of Washington, Seattle, WA; 2) Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Israel; 3) Department of Life Sciences, Bethlehem University, Palestinian Authority.

Nonsyndromic hearing loss (NSHL) is genetically heterogeneous. Most autosomal recessive NSHL is prelingual and severe to profound. Thus far, more than 30 autosomal recessive NSHL loci have been mapped and six genes have been cloned. We mapped recessive, prelingual, NSHL in a four-generation consanguineous Palestinian family. The maximum LOD score is 5.23 at the 6.5Mb interval on chromosome 22q13 bound by markers D22S1045 and D22S282. The DFNB28 region is contained completely within the much larger interval linked to DFNA17. DFNB28 is distal to the region deleted in velo-cardio-facial syndrome. Several candidate genes on chromosome 22q13 have been excluded as the cause of deafness in the family by sequence analysis. The DFNB28 region includes more than 70 genes, so ascertainment of distantly related affected individuals would be helpful to narrow the region.

Role of human norepinephrine transporter gene single nucleotide polymorphisms in cardiovascular disease. *M.K. Hahn¹, M.S. Mazei¹, D. Robertson^{2,3,5}, R.D. Blakely^{1,4}.* 1) Pharmacology; 2) Medicine; 3) Neurology; 4) Center for Molecular Neuroscience; 5) Autonomic Dysfunction Center, Vanderbilt University, Nashville, TN, 37232.

The norepinephrine transporter (NET) is responsible for clearance of norepinephrine (NE) from the synapse, recovering an estimated 90% of NE released from sympathetic nerves in the heart. NET is also a target for antidepressant drugs and psychostimulants. We recently reported a human NET (hNET; SLC6A2) coding mutation, A457P, in a proband with Orthostatic Intolerance (Shannon et al., NEJM, 2000). Genotyping of the proband's family by allele-specific oligonucleotide hybridization revealed that the mutant allele segregated with orthostasis-induced elevations in heart rate and plasma NE and decreases in the ratio of the NE metabolite, DHPG, to NE, suggestive of diminished NET activity. Indeed, we found that A457P results in near complete loss of [³H]NE transport to less than 2% of wild type (wt) activity in transfected cells. Biotinylation of cell surface proteins and western analysis reveal that the 80-100 kD form of hNET, the major species in the plasma membrane, is decreased in total cell extracts and in plasma membrane from COS-7 cells transfected with A457P compared to wt. Competition of [¹²⁵I]RTI-55 binding to membrane preparations demonstrates alterations in both antagonist and substrate binding to A457P. Cotransfection of A457P with wt hNET reveals a dominant negative interaction of decreased [³H]NE uptake to 59±2.4% of wt alone. Our laboratory, using WAVE DNA Fragment Analysis, and other groups (Stober et al., Amer J Med Genet, 1996; Halushka et al., Nat Genet, 1999) have identified additional hNET single nucleotide polymorphisms (SNPs). We are characterizing these SNPs and observe both loss of function as well as significant increases in transport in different mutants. By screening susceptible populations for hNET mutations and characterizing mutant proteins we hope to identify the structural components underlying transport function and illuminate the role and prevalence of hNET mutations in disease. Supported by MH58921 to R.D.B., S T32 HL07323 to M.K.H. and PO5 HL6693 to D.R.

Profibrillin maturation by human dermal fibroblasts: molecular chaperones in control and Marfan cell strains.

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Fibrillin-1, a major component of microfibrils in the extracellular matrix, is synthesized as profibrillin (profib), which undergoes proteolytic processing to the mature form. A processing site was localized to the C-terminal domain (C-term) at a PACE/furin consensus sequence. Although these proteases can be active in the trans-Golgi network (TGN), metabolic labeling studies have shown no evidence of intracellular processing of endogenously synthesized profib in dermal fibroblasts. Co-immunoprecipitation studies (co-IP) revealed that two ER-resident molecular chaperones, BiP and GRP94, interacted with profib. In addition, 38kDa and 36kDa proteins associated with profib, thus one of these proteins may act to prevent premature profib processing during secretion. Proteins of these sizes were associated with profib in 2 other human cell lines known to synthesize and process profib. These results imply that a cellular mechanism may prevent premature intracellular profib processing. We have identified 3 novel FBN1 mutations involving C-term in Marfan patient (MFS) dermal fibroblasts. Two of these patients have genomic mutations that cause aberrant splicing of exon 64, resulting in the production of a truncated profib molecule lacking the entire C-term. The third FBN1 mutation is a missense mutation in exon 63 altering the last EGF domain of the protein (R2680C). In addition, we have a patient cell line with a previously reported missense mutation in C-term (R2726W). The amount of p38 versus profib was determined using a Molecular Dynamics Storm 860 system. The results demonstrated that MFS fibroblasts with C-term mutations have one-half the amount of p38 associated with profib when compared with control cells or MFS fibroblasts with a missense mutation in the last EGF-like domain. These results imply that p38 binds to the C-terminal domain of profib. Co-IP studies of fibroblast cell lysates immunoprecipitated with polyclonal antibodies against type VI and type I collagen, fibronectin, and profib revealed that p38 and p36 were only present when profib was immunoprecipitated. This supports the hypothesis that p38 is a specific molecular chaperone for profib.

Identification of 20 full-length transcripts in the juvenile amyotrophic lateral sclerosis (ALS2) critical region at chromosome 2q33-q34: Candidate genes for ALS2. *S. Hadano*^{1,2}, *Y. Yanagisawa*², *J. Skaug*³, *K. Fichter*⁴, *S.W. Scherer*³, *G.A. Rouleau*⁵, *M.R. Hayden*⁴, *J.-E. Ikeda*^{1,2}. 1) The Inst. of Med. Sci., Tokai Univ., Kanagawa, Japan; 2) NeuroGenes Project, ICORP/JST, Tokai Univ., Kanagawa, Japan; 3) Dept. of Genet., The Hospital of Sick Children, Toronto, ON; 4) CMMT/Dept. of Med. Genet., Univ. of British Columbia, Vancouver, BC; 5) Centre for Res. in Neurosci., McGill Univ., Montreal, PQ.

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that manifests as selective upper and lower motor neuron degeneration. The autosomal recessive form of juvenile ALS (ALS2) has previously been mapped to the 1.7 cM interval flanked by D2S116 and D2S2237 spanning approximately 3 Mb region on human chromosome 2q33-q34. To understand the molecular mechanism for the pathogenesis of ALS2, identification of the gene and mutation that links to ALS2 is essential. To identify the transcribed DNA sequences mapped within the candidate region, we searched the public databases in GDB, Gene map 99 of the Human genome and UniGene at NCBI. Further, genomic DNA sequences derived from public database were used as quarries to detect ESTs. So far, 54 putative non-overlapping transcriptional units were assigned to this interval. Those units contained 20 full-length transcriptional units. Twelve of 20 genes were known (KIAA0005, CLK1, ORC2L, NDUFB3, CFLAR, CASP10, CASP8, UBL1, BMPR2, AIP-1, CD28, and CTLA4) and 8 genes were novel (CALS-109, CALS-7, CALS-C, CALS-21, CALS-69, CALS-117, CALS-135, CALS-79). Complete genomic organizations of 15 genes out of 20 full-length transcripts were defined by the computational analysis of cDNA and genomic DNA sequences. In addition, a total of 305 exons was detected in 54 transcriptional units. We analyzed the expression of transcripts by RT-PCR on total RNA extracted from normal human brain, spinal cord and lymphocyte. So far, 33 of 54 units have been analyzed for expression, and 32 of 33 units were expressed at least in the brain or spinal cord. Further analyses of the candidate genes represented by these units in the ALS2 patients are being performed to explore the genetic defect of ALS2.

Type I collagen $\alpha 1$ Gly 76-> Glu substitution in a child with type III OI causes increased susceptibility of the N-terminal end of the helix to enzymatic cleavage. *W.A. Cabral, E.J. Chernoff, J.C. Marini.* Section on Connective Tissue Disorders, NICHD/NIH, Bethesda, MD.

The majority of OI is caused by substitutions for glycine residues in type I collagen. Only 4% of possible nucleotide changes in the first two positions of collagen glycine codons would result in a glutamic acid substitution. Of the 190 independent nucleotide changes reported in type I collagen glycine codons, 7-8 glu substitutions would be expected. We describe here the clinical, biochemical and molecular characterization of a 13 year old girl with severe type III OI caused by a gly 76-> glu substitution in COL1A1. This is only the fourth report of a glutamic acid substitution in type I collagen.

Intact collagen chains and cyanogen bromide peptides from fibroblast collagen were electrophoretically normal, while proband osteoblast collagen was slightly overmodified. This suggested a mutation at the N-terminal end of the type I collagen helix. A mismatch was detected by RNA:DNA hybrid analysis in the region of cDNA coding for a portion of the N-propeptide through aa106 in the helical region. Subclones of both alleles were sequenced and revealed a G->A mutation causing an $\alpha 1(I)$ Gly 76-> Glu substitution in one allele. The mutation introduced a Tsp509I restriction site, which was used to confirm the presence of the mutation in the proband's leukocyte gDNA and its absence in parental gDNA. The glutamic acid substitution alters the structural and functional characteristics of the mutant collagen helices. Pericellular processing of type I collagen from proband fibroblasts showed a marked increase in the rate of N-propeptide removal as compared to control fibroblasts. Trypsin digestion of fibroblast collagen from the proband resulted in shortened $\alpha 1$ chains, as confirmed by CNBr analysis. The presence of the glutamic acid apparently exposes the adjacent Arg 75 residue in the $\alpha 1$ chain. In addition to altered local folding, the T_m for mutant helices from fibroblasts and osteoblasts was decreased 2-4°C versus controls, demonstrating a decrease in helix stability.

COL1A1 IVS A⁺ C mutation causes splicing out of exon 41 and confirms importance of this region for fibril self-assembly. *L.K. Green¹, W.A. Cabral¹, A. Fertala², J. Korkko², A. Forlino¹, J.C. Marini¹.* 1) Heritable Disorders Branch, NICHD NIH, Bethesda, MD; 2) Center for Gene Therapy, MCP Hahnemann University, Philadelphia, PA.

Previous data on the self-assembly of type I collagen into fibrils suggested that a crucial binding site maps to aa 776-797 of the $\alpha 1(I)$ chain. These experiments used synthetic peptides of sequences in the C-telopeptide. We confirmed the importance of this region for collagen assembly using a natural mutation with a deletion of aa 766-801 of the $\alpha 1(I)$ chain. This mutation occurs in a 5.5yr old boy with severe type III osteogenesis imperfecta. He is heterozygous for a COL1A1 IVS 41 A⁺ C substitution. Splicing out of exon 41 from proband cDNA was confirmed by sequencing of normal and shorter RT-PCR products. The mutant mRNA is more abundant in cells grown at 30°C than 37°C. SDS-urea-PAGE of proband type I collagen demonstrated the presence of structurally abnormal alpha chains in the cell layer but could not distinguish mutant chains in media. Cell layer findings included more rapidly migrating $\alpha 1(I)$ chains in 30° fibroblasts, overmodified $\alpha 1(I)$ chains (25% of total $\alpha 1(I)$ chains) in 37°C fibroblasts, and both fast and overmodified $\alpha 1(I)$ in 37°C osteoblasts. Thermal stability of proband collagen secreted by fibroblasts and osteoblasts were both decreased compared to control, supporting the presence of mutant helices in proband secreted collagen. Kinetics of binding of synthetic $\alpha 1$ C-telopeptide to secreted type I collagen collected from proband fibroblasts was analyzed with an optical biosensor. Altered on and off rates were observed in assays with proband collagen during bi-phasic binding of C-telopeptide. During the first stage of binding, peptide association with proband collagen was slower than with control, 4.29×10^6 vs 7.4×10^6 M⁻¹s⁻¹. Dissociation from proband collagen is faster than from control, 5.85×10^{-1} vs 1.17×10^{-1} s⁻¹. Thus, the overall affinity of the synthetic telopeptide is lower for proband. Changes in binding to proband sample may be caused by both the 36 residue deletion in mutant chain and altered conformation of proband normal $\alpha 1$ chains in helices containing a mutant chain.

Locus homogeneity in pseudoachondroplasia. *D.H. Cohn^{1,2}, L.M. King¹, S.L. Unger¹*. 1) Ahmanson Department of Pediatrics, Steven Spielberg Research Center, Cedars-Sinai Research Institute, Los Angeles CA, USA; 2) Departments of Pediatrics and Human Genetics, UCLA School of Medicine, Los Angeles CA, USA.

Pseudoachondroplasia (PSACH) (OMIM#177170) is a dominantly inherited disorder characterized by short limb dwarfism, loose joints, and early onset osteoarthritis. The diagnosis is based on clinical findings and typical radiographic changes consisting of anterior beaking of the vertebrae in childhood, small irregular epiphyses, and metaphyseal changes. Only mutations in the cartilage oligomeric matrix protein (COMP) gene have been reported in PSACH and all family studies have been consistent with linkage to the COMP locus on chromosome 19. COMP is a 524 kD homopentameric glycoprotein which is expressed mainly in cartilage but also in tendon and ligament. Each monomer has four distinct domains: a cysteine rich amino terminal domain that is responsible for pentamer formation, four epidermal growth factor-like domains, eight calmodulin-like (calcium binding) repeats, and a globular carboxyl terminus. To determine if all patients with PSACH have COMP mutations, we analyzed 25 consecutive patients by sequence analysis of all 19 COMP exons. In each case, a sequence change, either a missense mutation or a small in-frame deletion, was found. The majority of the mutations (24/25) were distributed among the exons which encode the calmodulin-like domains. One patient with classical PSACH had a mutation in the carboxyl terminal region. The COMP gene was also analyzed in four other patients, each with an unclassified form of spondyloepimetaphyseal dysplasia with some radiographic similarities to PSACH, and in one family with a severe form of SEMD. No COMP mutations were found in these patients. Our data, in conjunction with those from other groups, indicate that the typical PSACH phenotype is only produced by mutations in the COMP gene, that the mutations primarily occur in the exons of the gene which encode the calmodulin-like domain of the protein and that rare mutations in the exons that encode the carboxyl terminal region can also produce typical PSACH.

Leri Weill dyschondrosteosis caused by SHOX splicing mutation. *S.B. Flanagan¹, V.J. Hyland², C. Munns¹, M. Hayes¹, D. Vickers³, E. Rao⁴, G. Rappold⁴, J.A. Batch¹, I.A. Glass^{1,5}.* 1) Dept Paediatrics, University of Queensland, Brisbane, Queensland, Australia; 2) Dept Surgery, University of Queensland, Brisbane, Qld, Australia; 3) Dept Orthopaedics, Royal Children's Hospital, Brisbane, Qld, Australia; 4) Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany; 5) Queensland Clinical Genetics Service, Herston Hospitals, Brisbane, Qld, Australia.

Leri Weill dyschondrosteosis (LWD), a pseudo-autosomal dominant skeletal dysplasia, is characterised by mesomelic short stature and the Madelung deformity, a painful and disfiguring forearm abnormality of mid-childhood. Haploinsufficiency of a homeobox gene (SHOX; Short Stature Homeobox containing gene), that maps to the psuedoautosomal region of the X- and Y- chromosomes, has been shown to be the molecular basis of LWD. SHOX haploinsufficiency in LWD is usually due to large-scale deletions of the SHOX locus but two truncating mutations have also been identified. We report a 10.5 year old LWD female manifesting bilateral Madelung deformity with a pedigree consistent with (pseuedo) autosomal dominant inheritance and mesomelic short stature. The propoita was found by DNA sequencing to have a heterozygous point mutation in the splice donor site at the boundary of intron 2 and exon 3 of SHOX (IVS2-1G>A). This G to A transition leads to partial inclusion of intron 2 in the SHOX transcript due to activation of an intronic cryptic donor splice site, as demonstrated by detection of anomalous RT-PCR products, inclusive of intron 2 sequence. This mutation was found to co-segregate with the LWD phenotype. The previously reported nonsense mutations in SHOX result in truncated proteins that retain the homeodomain but lose the C-terminus. The IVS2-1G>A mutation is predicted to result in a severely truncated protein, lacking the homeodomain and thus, DNA binding properties. Quantitative RT-PCR is underway to determine the extent of expression-level SHOX haploinsufficiency in bone marrow fibroblasts from this patient, compared to SHOX-deleted LWD cases and non-LWD Madelung cases. This mutation is novel and is the first splicing mutation to be discovered.

Domain-Specific Mutations in the Human Transforming Growth Factor Beta 1 Gene (TGFB1) Result in Camurati-Engelmann Disease. A. Kinoshita¹, T. Saito², M. Ghadami¹, H. Tomita¹, Y. Makita¹, K. Yamada¹, S. Ikegawa³, G. Nishimura¹, Y. Fukushima⁴, J.C. Murray⁵, N. Taniguchi², N. Niikawa¹, K. Yoshiura¹. 1) Department of Human Genetics, Nagasaki University School of Medicine; 2) Department of Biochemistry, Osaka University Medical School; 3) Human Genome Center, Institute of Medical Science, University of Tokyo; 4) Department of Medical Genetics, Shinshu University School of Medicine, Japan; 5) Department of Pediatrics and Biological Science, University of Iowa.

The TGF-beta superfamily consists of multiple members with a range of biological activities that regulate cell growth and differentiation across many cell types. We previously assigned the locus for Camurati-Engelmann disease (CED, MIM b131300) to a 15.1-cM region at chromosome 19q13.1-q13.3. By a positional candidate gene approach, we found three different missense mutations (R218H, R218C, and C225R) near the C-terminus of the latency associated peptide (LAP) of latent TGF-beta1 in nine CED families (seven unrelated Japanese and two Caucasian families). Analysis of TGF-beta1 secretion indicated that these mutations cause conformational instability of LAP and disrupt the interaction between LAP and TGF-beta1. These findings not only explain the pathogenesis of CED, but also provide evidence that TGF-beta1 plays an important role in modeling and remodeling regulation of the diaphysis of long bones. It further supports the notion that domain-specific mutations in widely expressed genes can result in unique pathophysiology that can suggest previously unsuspected avenues for treatment and functional studies.b.

Identification of two previously undescribed patients with familial expansile osteolysis who have mutations in the TNFRSF11A signal peptide. *R.J. Leach^{1,2}, B.E. Reus¹, F.R. Singer³, H.G. Bone⁴, M.J. Nellisery⁵, M.F. Hansen⁵, T.L. Johnson-Pais².* 1) Cellular & Structural Biol, Univ Texas Health Sci Ctr, San Antonio, TX; 2) Pediatrics, Univ Texas Health Sci Ctr, San Antonio, TX; 3) John Wayne Cancer Institute, Santa Monica, CA; 4) Michigan Bone & Mineral Clinic, Detroit, MI; 5) Center Molecular Medicine, Univ of Conn Health Ctr, Farmington, CT.

Familial expansile osteolysis (FEO) is a rare, autosomal dominant genetic disorder, which is characterized by abnormal bone remodeling. Recent studies by Hughes et al. (2000) identified 3 independent FEO families who all had 18 bp in frame insertion mutations in the first exon of the TNFRSF11A gene (a.k.a. RANK). The resulting mutation occurs in the signal peptide and causes an increase in RANK-mediated NF-kB signaling, consistent with a gain-of-function mutation. We have identified two additional FEO patients. One has no family history of FEO but presented with classic symptoms including bilateral hearing loss at an early age, deterioration of teeth by the late 20's, and severe pain and swelling in distal tibia before the age of 30. The second patient had extensive expansile tibial lesion, which was first noted in adolescence, and less prominent lesions in one humerus and a phalanx. Her hearing deteriorated after age 10 as did her teeth in her 20's. Her father also had skeletal involvement, hearing loss, and loss of dentition. Mutational analysis of the TNFRSF11A gene in these patients demonstrated an 18 base pair insertion in the first exon, in the exact location identified by Hughes et al. (2000). In addition, a single base pair change was observed in the base preceding the insertion in both of our patients when compared to the published sequence. The base pair change, however, was in the third position of the codon and would have no effect on amino acid sequence. These results demonstrate the highly specific nature of the mutations in the TNFRSF11A gene that give rise to FEO.

A mild skeletal dysplasia with acanthosis nigricans due to a K650Q mutation in the FGFR3 gene. *G.R. Mortier¹, L. Nuytinck¹, J. Lambert³, J.M. Naeyaert³, J.G. Leroy^{1,2}*. 1) Department of Medical Genetics, Ghent University Hospital, Ghent, Belgium; 2) Department of Pediatrics, Ghent University Hospital, Ghent, Belgium; 3) Department of Dermatology, Ghent University Hospital Ghent, Belgium.

Mutations in the FGFR3 gene can result in several bone disorders characterized by various combinations of dwarfism, craniosynostosis and rarely acanthosis nigricans. Mutations in codon 650 have been reported in thanatophoric dysplasia type II (K650E) and SADDAN (severe achondroplasia with developmental delay and acanthosis nigricans) (K650M). We have identified a different mutation K650Q, resulting in a mild skeletal dysplasia with acanthosis nigricans. The female proband, born with a length of 48 cm, was referred at the age of 10 months because of short stature. Height was 63 cm (P3=66cm) and OFC 46.3cm (P75-97). Short limbs with redundant skin folds and a mild thoracolumbar gibbus were noted. A skeletal survey excluded the diagnosis of achondroplasia but revealed short and thick tubular bones with small epiphyses, short iliac wings and normal spine. On follow-up, the psychomotor development was normal. At 8 years the height was 107 cm (P3=116cm), the span 108 cm and the OFC 54 cm (P98). Fascinating was the new finding of acanthosis nigricans (proven histologically) in both flanks and axillae, which according to the parents started at the age of 5 years in the neck. On skeletal radiographs the tubular bones were short but the diaphyses less widened than before. In addition, small carpal bones, normal vertebral bodies with normal lumbar interpediculate widening, slightly shortened femoral necks and small iliac wings were observed. The association of acanthosis nigricans with a skeletal dysplasia prompted us to perform mutation analysis of the FGFR3 gene. Heterozygosity for a A to C transversion (first nt) in codon 650, implying the K650Q substitution, was detected in the proband. The clinical and radiographic phenotype of this patient resembles best hypochondroplasia. This report confirms that mutations in codon 650 of the FGFR3 gene can cause acanthosis nigricans in association with a skeletal dysplasia even expressed as mere proportionate short stature.

Cartilage expressed sequence tags provide insight into chondrocyte gene expression and identify novel candidate genes for the osteochondrodysplasias. *D. Krakow¹, E.T. Sebald¹, L.M. King², D.H. Cohn^{2,3}*. 1) Dept of Ob/Gyn and Division of Medical Genetics, Cedars-Sinai Medical Center, UCLA School of Medicine; 2) Department of Pediatrics and Division of Medical Genetics, Cedars-Sinai Medical Center, UCLA School of Medicine; 3) Department of Human Genetics, UCLA School of Medicine, Los Angeles, California.

The osteochondrodysplasias are a heterogeneous group of approximately 150 disorders that result in abnormal skeletal development. Recent progress has been made in identifying a number of loci and disease genes for these phenotypes. However because of their rarity and small family size, many of the remaining disorders are not amenable to traditional genetic analysis. To identify new candidate genes for the osteochondrodysplasias and to better characterize the transcriptional profile of chondrocytes, we constructed and analyzed an unsubtracted fetal cartilage cDNA library. A total of 376 clones were selected for sequence analysis. The sequences of 293/376 clones matched 163 known human genes: defects in several of these produce osteochondrodysplasias. Although some of the isolated genes are expressed in multiple other tissues, our data establish their expression in cartilage. Some of these genes come from families where other gene members when mutated produce skeletal phenotypes. We identified 3 clones from the zinc finger gene family, ZNFs 216, 264 and 83 and thereby identify other members of the family that may have a regulatory role in cartilage. Osteonectin/SPARC whose expression is known to be in cartilage and produces a skeletal phenotype in homozygous $-/-$ mice represented 2% of the clones, strongly suggesting it as candidate for a human osteochondrodysplasia. 73 of the remaining clones (22%) matched ESTs of unknown identity that were expressed in multiple tissues. There were 10 clones with no significant database matches that represent novel genes expressed in cartilage. The emerging genome sequence has allowed the chromosomal location, as well as gene structure for some of these genes to be established, and provides a means to analyze their involvement in specific osteochondrodysplasias.

Genetic heterogeneity in Leri-Weill dyschondrosteosis. *C. Huber, V. Cusin, N. Dagoneau, M. Le Merrer, A. Munnich, V. Cormier-Daire.* Department of Genetics and INSERM U393, Hospital Necker, Paris, France.

Dyschondrosteosis (DCS) has been recently ascribed to large scale deletions or non sense mutations in the SHOX gene. Studying 12 novel DCS families, we found linkage to the Pseudoautosomal Region (PAR) in 10/12 and large scale deletions in 2/10 families. By directly sequencing the SHOX coding region, we were able to detect distinct point mutations which cosegregated with the disease in 5/12 families. The mutations resulted in premature stop codons in 4 families. In the fifth family, a C®T transition in exon 3 resulted in an aminoacid change (A173C) in the homeodomain, which is highly conserved across species. Molecular analyses failed to detect mutation in 3/10 families. Finally, linkage analyses using an intragenic CA-repeat excluded SHOX as the disease gene in 2/12 families. Interestingly, no significant clinical difference between families linked or unlinked to SHOX were noted and a intrafamilial variability was frequently observed. This study gives additional support to the view that haploinsufficiency of SHOX is responsible for the majority of DCS cases (7/12). It also shows that point mutations in the SHOX gene are not so rare (5/12). Finally, the exclusion of the SHOX gene in two families is highly suggestive of a genetic heterogeneity. Extensive genome wide search in these families will hopefully help identifying other disease genes in DCS.

DTDST is expressed in developing fetal cartilage but also in a wide variety of other tissues and cell types. S.

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The diastrophic dysplasia sulfate transporter (DTDST) gene is responsible for the family of three chondrodysplasias, namely diastrophic dysplasia (DTD), atelosteogenesis type II (AO2), and the lethal achondrogenesis type IB (ACG1B). The severity of the clinical phenotype correlates with the DTDST mutation and residual function retained by the defective protein. DTDST acts as a sulfate/chloride antiporter and belongs to the recently recognized anion transporter gene family (Lohi et al. this meeting). Other fully characterized members are the major intestinal chloride/bicarbonate exchanger CLD/DRA that causes congenital chloride diarrhea, PDS, a chloride/formate exchanger that causes Pendred syndrome, and recently cloned Prestin and PAT1. Although the phenotype caused by DTDST mutations suggests cartilage as the major expression site, Northern analysis shows ubiquitous expression pattern with weak basal expression in almost all tissues. In situ hybridization study of normal human colon revealed abundant DTDST expression.

We have used in situ hybridization and immunohistochemistry to search multiple normal tissues for the expression of the human DTDST gene and protein. As expected, a strong signal for DTDST mRNA was detected in developing fetal finger cartilage, while in adult only bronchial cartilage showed expression. Abundant DTDST mRNA expression could also be detected in eccrine sweat gland and in bronchial glands, as well as a weaker signal in liver parenchyme and placental villi. Immunoreactivity for the DTDST protein was observed in addition also in exocrine pancreas.

Characterization of tissues and specific cell types that express DTDST in vivo is important in elucidating physiological function of the normal protein and the pathophysiology of DTD, AO2, and ACG1B.

A multiplicity of loci for multiple epiphyseal dysplasia. *S.L. Unger¹, L.M. King¹, D. Sobetzko², A. Superti-Furga², D.H. Cohn^{1,3}.* 1) Ahmanson Department of Pediatrics, Steven Spielberg Pediatric Research Center, Cedars-Sinai Research Institute, Los Angeles CA, USA; 2) Division of Metabolic and Molecular Diseases, Department of Pediatrics, University Children's Hospital, Zurich Switzerland; 3) Departments of Pediatrics and Human Genetics, UCLA School of Medicine, Los Angeles CA, USA.

Multiple epiphyseal dysplasia (MED) is a relatively mild osteochondrodysplasia characterized by normal to mild short stature, early onset joint degeneration and small dysplastic appearing epiphyses on radiographs. The first MED locus identified was the cartilage oligomeric matrix protein (COMP) gene (EDM1, OMIM#132400). Subsequently, mutations in the COL9A2 and COL9A3 genes of type IX procollagen, all leading to skipping of exon 3, were found in several MED patients (EDM2, OMIM#600204 and EDM3, OMIM#600969). EDM1, 2 and 3 are inherited in an autosomal dominant manner. Recently, mutations in the diastrophic dysplasia sulfate transporter (DTDST) gene have been shown to cause a recessive form of MED (EDM4, OMIM# 226900). To date, mutations have been reported in 19 MED probands and the majority (12) have been COMP mutations. Six mutations have been reported in either COL9A2 or COL9A3 and one at the DTDST locus. We screened 25 consecutive MED patients for mutations at these loci and at COL9A1, which encodes the third chain of type IX procollagen. We identified 7 (28%) COMP mutations; six predicted to cause an amino acid substitution within the calmodulin repeat domains of the protein. Two patients were homozygous for the R279W mutation in DTDST. No changes were detected at the type IX procollagen genes. Our results indicate that although COMP may be the major MED locus it still accounts for only a minority of MED cases. Mutations in the type IX procollagen genes and DTDST appear to be less frequent causes of MED. The diversity of loci identified and the small proportion of MED patients accounted for by these loci suggest that this relatively mild phenotype could be produced by defects in many additional components of the cartilage extracellular matrix. MED may thus be among the most genetically heterogeneous of the osteochondrodysplasias.

Characterization of myosin type X (MYO10), a candidate gene for familial chondrocalcinosis. *C.J. Williams¹, R. McGrath¹, E. Johnson², J. Overhauser²*. 1) Dept Medicine/Rheumatology, Thomas Jefferson Univ, Philadelphia, PA; 2) Dept Biochemistry/Molecular Pharmacology, Thomas Jefferson University, Philadelphia, PA.

Familial chondrocalcinosis is an autosomal dominantly inherited arthropathy that is characterized by the deposition of calcium-containing crystals in the joint space, often followed by the development of severe osteoarthritis. Several families have been utilized for linkage analyses and the gene for the disorder resides on chromosome 5p in the region of the polymorphic marker D5S416. Physical and transcript mapping of the interval have revealed several transcripts that may be considered potential candidate genes for the disease. Among these is myosin type X (MYO10), a member of the family of unconventional myosins. We have characterized MYO10 in order to evaluate it as the chondrocalcinosis gene. MYO10 spans approximately 200Kbp of genomic sequence. It contains 41 exons that range in size from 15bp to 876bp. Several introns contain multiple repetitive elements, including members of the ALU and LINE families. The gene encodes a 6.8Kbp transcript resulting in a 2,058 amino acid protein. The protein contains a head region composed of an ATP- and actin-binding motor domain, a neck region that consists of a coiled-coil domain for dimerization, and a tail region that presumably binds molecular cargo for cellular transport. The transcript is expressed in a variety of tissues including adult articular cartilage. We have begun mutation analysis of the MYO10 gene in the three chondrocalcinosis families that we have used for linkage analyses. PCR primers for amplification of family DNA samples were derived from cosmid- and BAC-generated genomic sequence. To date, these studies have detected numerous intronic base substitutions, synonymous polymorphisms, and several non-synonymous polymorphisms including an H \rightarrow Y in exon 4, an R \rightarrow W in exon 11, a D \rightarrow N in exon 19, an L \rightarrow V in exon 35, and an S \rightarrow T in exon 36. In one of our families, we have detected a splice donor sequence variation in intron 6 that is followed by a deletion of 4bp. Analysis of this apparent polymorphism is underway to determine if it effects downstream splicing reactions. (Supported by NIH R01 AR44360).

Investigation of the potential role of RANK and OPG in Paget's disease of bone. *W. Wuyts¹, L. Van Wesenbeeck¹, A. Morales-Piga², S. Ralston³, A. Hughes⁴, D. Anderson⁵, F. Vanhoenacker¹, W. Van Hul¹.* 1) Dept Medical Genetics, Univ Antwerp, Antwerp, Belgium; 2) Dept Rheumatology, Hospital Ramon Y Cajal, Madrid, Spain; 3) Dept Medicine and Therapeutics, University of Aberdeen, Aberdeen, UK; 4) The Queen's University, Belfast, UK; 5) Immunex Corporation, Seattle, USA.

Paget's disease of bone (PDB), one of the most common bone disorders in the Western world, is a localized disorder of bone. The process is initiated by an increase in osteoclast mediated resorption, which is then followed by increased bone remodeling resulting in the formation of poorly structurized bone. One of the main pathways influencing osteoclast formation and activation involves the binding of RANKL to RANK resulting in proliferation and differentiation of osteoclast precursors while OPG acts as an inhibitor of osteoclastogenesis by serving as a decoy receptor for RANKL. Recently, mutations in the RANK gene have been shown to cause Familial Expansile Osteolysis, a rare bone disorder showing great similarity with PDB. We have now performed mutation analysis in the RANK and OPG genes in 36 PDB patients to investigate whether mutations in these genes could be responsible for PDB. Our data suggest that RANK is not directly involved in PDB in our set of patients, as no mutations in the RANK coding region could be identified and allele frequencies of RANK polymorphisms did not differ in PDB patients compared to the random population. SSCP and sequencing analysis of the OPG gene in the same set of 36 PDB patients did not reveal any disease causing mutations but several polymorphisms were identified. For each polymorphism we determined allele frequencies in our set of PDB patients and compared them to the control population. Interestingly, preliminary results show that for one intronic C/T polymorphism the frequency of the C allele is statistically increased in PDB patients suggesting an association of this allele with PDB. At present a larger set of PDB patients is being analyzed to determine whether people harbouring this allele may be more susceptible for developing PDB.

A novel autosomal dominant basal ganglia degeneration with cavitation on 19q can mimic Parkinson's disease and Huntington's disease. *J. Burn*¹, *A.R. Curtis*¹, *L. Bindoff*^{2,5}, *P. Ince*², *A. Coulthard*², *A. Jackson*³, *M. Jackson*², *C. Fey*¹, *D. Hay*¹, *D. Shepherd*⁴, *D. Bates*², *A. Curtis*¹. 1) School of Biochemistry and Gen, Univ Newcastle on Tyne, Newcastle on Tyne, England; 2) Neurology, Neuropathology, Radiology, Newcastle Hospitals Trust; 3) Medical Genetics, St James Hospital Leeds; 4) St Mary's Hoapital, Manchester; 5) Haukeland Sykehus, Bergen, Norway.

An extensive pedigree in North West England contains multiple individuals affected by an apparently fully penetrant but highly variable late onset degeneration of the basal ganglia which can mimic Huntingtons disease and Parkinsons disease but is not associated with significant dementia. Chorea, athetosis, spasticity, rigidity and bulbar dysfunction are described in various affected relatives. Late stage disease is characterised by dramatic cavitation of the basal ganglia. To date no evidence of a trimeric repeat disorder is apparent on molecular or immunohistochemical analysis and there is no clear evidence of anticipation. A tissue distribution of disease reminiscent of DRPLA was found but with unique histology involving neuroaxonal spheroid formation. Genomic mapping has localised the causative gene to 19q with a lod score of 3.5 at a theta of zero. This excludes all similar late onset degenerative neurological disorders. An obligate carrier was born in 1840 and family reports suggest the disease was recognised in a founder Cumbrian family, the Fletchers, in the late 18th century. In addition to possible links to migrants to the United States, one member, Fletcher Christian, achieved fame as the leader of the mutiny on the Bounty. There were no reports of a similar disease in the colony that he founded on the Pitcairn Islands. This group was later dispersed in the Pacific area. The variable late onset phenotype makes it possible that other affected descendants could occur in that region.

Comprehensive mutation analysis and genotype/phenotype correlations in a cohort of 227 tuberous sclerosis patients. *S.L. Dabora¹, P.S. Roberts¹, J. Chung¹, Y.S. Choy¹, F. Hall¹, A. Nieto¹, M.P. Reeve¹, D. Franz², S. Jozwiak³, E. Thiele⁴, D.J. Kwiatkowski¹.* 1) Division of Hematology, Brigham & Women's Hospital., Boston, MA; 2) Division of Pediatric Neurology, Children's Hospital Medical Center, Cincinnati, OH; 3) Department of Child Neurology, Children's Memorial Hospital, Warsaw, Poland; 4) Children's Hospital, Boston, MA.

We have developed a comprehensive strategy for mutation detection in tuberous sclerosis complex (TSC) which includes exon by exon analysis of TSC2 and TSC1 using denaturing high performance liquid chromatography (DHPLC) to identify small insertions/deletions (indels) and point mutations (Choy et al., 1999; 63:383-391). This is followed by long range PCR and quantitative PCR assays to identify large deletions in TSC2. These methods are being used to identify mutations in a cohort of 227 TSC patients (86%; sporadic cases and 14%; familial cases). In total, mutations have been identified in 169/227 (74%;) patients. This includes 142 TSC2 mutations and 27 TSC1 mutations. In TSC1, there are 11 nonsense mutations, 15 indels, and 1 missense mutation. In TSC2, there are 35 nonsense mutations, 27 small frameshift indels, 12 in-frame deletions, 27 splice site mutations, 27 missense mutations, 13 large deletions and 1 large insertion. Seven of the TSC2 large deletions and one large insertion were identified using long range PCR (Dabora et al., J. Med. Genet., in press). The remaining 6 were identified using a quantitative multiplex PCR assay. Genotype/phenotype analysis has been done in sporadic cases of TSC for which full clinical information is available: 13 patients with TSC1 mutations and 53 patients with TSC2 mutations. Sporadic TSC patients with TSC1 and TSC2 mutations have similar frequencies of seizures, hypomelanotic macules, subependymal nodules, subependymal giant cell astrocytomas, facial angiofibromas, renal cysts, and renal angiomyolipomas. There was a small difference in the incidence of mental handicap in the two groups (6/11 TSC1 patients and 33/43 TSC2 patients), but this was not statistically significant. In summary, TSC2 mutations are much more common than TSC1 mutations, but seem to be associated with similar clinical phenotypes.

Comprehensive screening of TOR1A (DYT1) gene in early-onset dystonia patients: no evidence of allelic heterogeneity. *L. Cavalier¹, S. Carles¹, C. Guittard¹, A. Roubertie², B. Echenne², P. Coubes³, M. Claustres¹, S. Tuffery-Giraud¹.* 1) lab Genetique Moleculaire, Montpellier, France; 2) Service de Neuro-Pediatrie; 3) Service de Neurochirurgie CHU de Montpellier, France.

Idiopathic torsion dystonia (ITD) represent a clinically and genetically heterozygous group of movement disorders. The most frequent form is early-onset generalized dystonia, beginning in childhood and inherited in an autosomal-dominant mode with reduced penetrance (30-40%). Most cases are caused by a single mutation, a 3-bp deletion (946delGAG) in the TOR1A gene in 9q34. We examined 49 unrelated patients with early-onset ITD for the 946delGAG mutation: 37% (14/49) were found to carry the GAG deletion, all with TOR1A typical phenotype and age of onset ranging from 6 to 9 years. This result confirms previous findings that the TOR1A gene does not underlie ITD in all families. The high proportion of Caucasian ITD patients with early-onset dystonia phenotype who do not carry the mutation may represent either allelic or locus heterogeneity. In order to identify other mutations, we analysed the TOR1A cDNA in all affected individuals tested negative for the GAG deletion. Using total RNA isolated from patients peripheral blood lymphocytes, the whole coding sequence was amplified by RT-PCR and screened for mutations by sequence analysis. No additional mutation could be found in the TOR1A gene of patients who have been analysed. Thus, the 946delGAG seems to be the unique mutation in the TOR1A gene, excluding the allelic heterogeneity hypothesis in our series. The predominance of this mutation may reflect genetic instability in an imperfect tandem 24-bp repeat in the region of the deletion. Furthermore, we could underline that 17% (6/35) of patients presented with age of onset below 5 years, suggesting the involvement of another locus for a very early-onset generalized dystonia phenotype. In order to demonstrate locus heterogeneity, familial cases of early-onset dystonia should be available for genotype analysis. This work was partly supported by a grant from the Association Sophie.

Hereditary Spastic Paraplegia caused by Mutations in the SPG4 Gene. *J.J. Burger¹, N. Fonknechten², M. Hoeltzenbein³, L. Neumann¹, J. Hazan², A. Reis¹.* 1) Charite Human Genetics , Humboldt Univ, Berlin, Germany; 2) Genoscope, Evry, France; 3) Institute of Human Genetics, Ernst-Moritz-Arndt-Universitt, Greifswald, Germany.

Autosomal dominant hereditary spastic paraplegia (AD-HSP) is a genetically heterogeneous neurodegenerative disorder characterised by progressive spasticity of the lower limbs. The SPG4 locus at 2p21-p22 accounts for 40-50% of all AD-HSP families. The SPG4 gene was recently identified. It is ubiquitously expressed in adult and foetal tissues and encodes spastin, an ATPase of the AAA family. We have now identified four novel SPG4 mutations in German AD-HSP families, including one large family for which anticipation had been proposed. Mutations include one frame-shift and one missense mutation, both affecting the Walker motif B. Two further mutations affect two donor splice sites in introns 12 and 16, respectively. RT-PCR analysis of both donor splice site mutations revealed exon skipping and reduced stability of aberrantly spliced SPG4 mRNA. All mutations are predicted to cause loss of functional protein. In conclusion, we confirm in German families that SPG4 mutations cause AD-HSP. Our data suggest that SPG4 mutations exert their dominant effect not by gain of function but by haploinsufficiency. If a threshold level of spastin would be critical for axonal preservation, such threshold dosage effects might explain the variable expressivity and incomplete penetrance of SPG4-linked AD-HSP.

Exclusion of RNX as a major gene in Congenital central hypoventilation syndrome (CCHS, Ondine's curse). J.

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CCHS (MIM 209880) is an hitherto unexplained disorder of the metabolic control of breathing leading to life-threatening episodes starting mostly from birth. While the majority of cases is sporadic, several familial forms have been reported and segregation analyses have suggested that the multifactorial and the major locus models are almost equally likely in CCHS. Interestingly, the CCHS-Hirschsprung disease association has been frequently reported (Haddad syndrome, MIM 209880) and mutations of the RET-GDNF and the endothelin pathways have been reported in rare cases of CCHS and Haddad syndrome. HOX11 genes constitute a family of orphan homeobox genes. Recently the Rnx (respiratory neuron homeobox, Hox 11L2) knock-out mice was described and Rnx ^{-/-} mice die within 24 hours after birth from apnea and cyanosis, while transient spontaneous respiration is possible upon strong stimulation. This phenotype, together with the Rnx expression in the medulla oblongata, prompted us to consider the RNX gene as a candidate for CCHS in human. A total of 30 patients were included in the study (26 CCHS patients and 4 Haddad syndrome cases). All patients fulfilled the inclusion criteria for diagnosis of CCHS, namely : i) hypoventilation, hypoxaemia and hypercapnia especially during quiet (NREM) sleep on polysomnographic respiratory recording, with ii) no cardiac, pulmonary, neuromuscular, EEG or cerebral MRI anomaly. SSCP was used to screen the RNX gene for nucleotidic variation in the coding sequence. An intronic polymorphism (I1 421+34C->T) was found in 8 CCHS cases and 2 Haddad syndrome cases, excluding a deletion of the region in this patients. Non significant variations from the reported sequence were also found in patients and controls. We therefore conclude that RNX is not a major gene predisposing to CCHS in human. BDNF (brain-derived neurotrophic factor), and MASH 1, a neural-specific bHLH transcription factor, remain good candidate genes in this disease and are currently under investigation.

Five novel mutations of spastin gene in chromosome 2-linked autosomal dominant spastic paraplegia (SPG4). *H-X. Deng, H. Zhai, W. Chen, W-Y. Hung, A. Hentati, T. Siddique.* Neurology Dept, Northwestern Univ, Chicago, IL.

Autosomal dominant hereditary spastic paraplegia (AD-HSP) is a group of genetically heterogeneous neurodegenerative disorders characterized by progressive spasticity of the lower limbs. Eight AD-HSP loci have been mapped to chromosomes 14q (SPG3), 2p (SPG4), 15q (SPG6), 8q (SPG8), 10q (SPG9), 12q (SPG10), 19q (SPG12) and 2q (SPG13). The SPG4 locus at 2p21-p22 has been shown to account for approximately 40% of all AD-HSP families. Mutations in a putative nuclear AAA protein have recently been identified to be responsible for chromosome 2-linked SPG4. There are altogether 39 mutations identified. These mutations are distributed in entire SPG4 gene except exons 1, 4 and 6. By SSCP and sequencing analysis, we have identified 5 novel mutations in SPG4 gene. They are E112STOP in exon 1, Y237STOP in exon 4, R399STOP and S404F in exon 9, and G527D in exon 14.

DHPLC analysis of unrelated CMT patients in the Myotubularin related 2 gene, *MTMR2*, responsible for Charcot-Marie-Tooth disease type 4B. A. Bolino¹, L. Lonie¹, M. Zimmer¹, C.F. Boerkoel², A.P. Monaco¹, J.R.

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Charcot-Marie-Tooth type 4B (CMT4B) represents an autosomal recessive demyelinating neuropathy characterised by infantile onset with progressive symmetric distal and proximal weakness starting in the lower extremities, cranial nerve involvement in most of the cases, and focally folded myelin sheaths in the peripheral nerve. We recently demonstrated that CMT4B is caused by mutations in the gene encoding myotubularin related protein 2, a dual specificity phosphatase, *MTMR2*, on chromosome 11q22. Five different loss of function mutations, all of them in homozygosity, were demonstrated in unrelated CMT4B patients. *MTMR2* is the fifth gene identified as responsible for a CMT disorder and the first gene encoding a phosphatase to be associated with a peripheral neuropathy. To estimate the frequency of mutations in *MTMR2*, we undertook a screening of 183 unrelated CMT patients, using the Denaturing High Performance Liquid Chromatography (DHPLC). These patients have been found to be negative for mutations occurring in the known genes responsible for CMT. After analysing the 15 coding exons of the *MTMR2* gene in a first set of 95 patients, we found a missense mutation in exon 15 (1805C->G, Ala602Gly) in a patient with a Congenital Hypomyelinating Neuropathy, CHN. This change was not detected on 392 normal chromosomes analysed. In addition, we demonstrated two high frequency polymorphisms, 8C->A, Thr3Lys (allele frequencies 61% and 39%) and 1131T->C, Thr377Thr (allele frequencies 64% and 36%), in exons 1 and 10, respectively. Three rare polymorphisms in exon 4, 298G->A, Ala100Thr (2/392 chromosomes, 0.5%); in exon 11, 1233 G->A, Thr411Thr (2/190 chrs, 1%); and in exon 13, 1504G->C, Glu502Gln (8/392 chrs, 2%) were also detected. The finding of a heterozygous missense mutation in a patient with CHN is suggesting that *MTMR2* can act through different biological mechanisms in causing CMT disorders.

Parkin gene in idiopathic Parkinson's disease. *G. Annesi¹, F. Annesi¹, R.L. Oliveri², I.C. Ciro' Candiano¹, A.A. Pasqua¹, P. Spadafora¹, D. Civitelli¹, E.V. De Marco¹, C. Tomaino¹, M. Zappia², A. Quattrone^{1,2}.* 1) Inst Exp Med and Biotech, National Research Council, Mangone, Cosenza, Italy; 2) Inst Neurol, Dept Med Sci, Univ Catanzaro, Italy.

A frequent cause of autosomal-recessive juvenile parkinsonism (AR-JP) are point mutations and deletions of the parkin gene. AR-JP has been described in Japanese, European, and North African families. In European families the identified mutations were associated with a wide range of ages at onset as well as of clinical signs. Thus, it has been hypothesized that in many patients the phenotype of AR-JP may be clinically indistinguishable from idiopathic Parkinson's disease (PD). Furthermore, recent reports showed that polymorphisms located in the parkin gene are associated with sporadic PD in Japanese population. To clarify whether the parkin gene is a susceptibility locus in classical late-onset PD, we sequenced the 12 exons of the gene in 15 families with recessive inheritance of PD and in 100 sporadic cases of PD. Furthermore we studied in these patients and in population controls the four polymorphisms located in exons 4, 10 and 11 of the gene. The results showed no mutations in the parkin gene either in familial or in sporadic PD cases. The frequencies of the four polymorphisms investigated were not different among familial PD, sporadic PD and population controls. Our data do not support that the parkin gene is a major locus for classical late-onset PD, both sporadic and familial, at least in Caucasian population.

Characterization of the BEM 46 gene, a candidate for the mouse deafness (*dn*) and human DFNB7/DFNB11

genes. *D. Arnaud*¹, *S. Drury*¹, *D. Scott*³, *R. Poche*¹, *R. Sundstrom*³, *Z. Den*¹, *V. Sheffield*³, *M. Batzer*¹, *P. Deininger*², *M. Lovett*⁴, *R. Smith*³, *B. Keats*¹. 1) Louisiana State Univ. Health Sciences Center, New Orleans, LA; 2) Tulane Medical Center, New Orleans, LA; 3) University of Iowa, Iowa City, Iowa; 4) University of Texas Southwestern Medical Center, Dallas, Texas.

The deafness (*dn*) mouse is the result of a spontaneous mutation in the curly tail (*ct*) stock. Homozygous *dn/dn* mice have sensorineural hearing loss with degeneration of the Organ of Corti and the stria vascularis. The *dn* gene is likely to be the orthologue of the autosomal recessive nonsyndromic loci DFNB7/DFNB11 on human chromosome 9. The *dn* locus was mapped by linkage analysis to mouse chromosome 19 and the recombination frequencies with markers in the region indicated the presence of an inversion, with one of the breakpoints located between D19Mit14 and D19Mit96. A BAC contig of this 200 kb region was constructed and two novel cochlear expressed genes, ZNF216 and TMEM2, were mapped to it. However, both were excluded as the *dn* gene. We have recently identified another cochlear-expressed gene that maps to both the DFNB7/DFNB11 and *dn* intervals. This gene shares significant homology with the uncharacterized yeast gene BEM 46, a temperature-sensitive suppressor of the yeast genes BEM1/BUD 5, which function in cell polarity, bud emergence and the actin cytoskeleton. We have determined the cDNA sequence for both the mouse (2.2kb) and human (2.4kb) genes. The gene is composed of two exons; the ORF is about 700 bp and it encodes a 284 amino acid protein. To determine if BEM 46 might be the *dn* gene, we sequenced *dn/dn* and *ct/ct* RNA. No differences were observed. Northern blot analysis indicated that expression was highest in the lung and brain, and ESTs from this gene were detected in libraries constructed from mouse lymph node, mammary gland, hypothalamus, heart, uterus, liver, placenta, and testis, in addition to cochlea. Using RT-PCR and northern blot analyses, we have not detected any changes in transcript size or quantity between *dn/dn* and *ct/ct* mice. Thus, it is unlikely that a mutation in BEM 46 is responsible for the deafness phenotype in the *dn* mouse.

CACNA1A Gene Polymorphisms in Families With Episodic Ataxia and Hemiplegic Migraine. *I. Alonso¹, J. Coelho¹, P. Mendonça¹, L. Jardim², A. Tuna³, J. Barros³, P. Coutinho⁴, J. Sequeiros^{1, 5}, I. Silveira¹.* 1) Unigene, IBMC - Univ Porto, Porto, Portugal; 2) Hospital Clínicas de Porto Alegre, Brazil; 3) HGSA, Porto, Portugal; 4) Hospital de São Sebastião, Feira, Portugal; 5) ICBAS - Univ Porto, Porto, Portugal.

Episodic ataxia type 2 (EA-2), familial hemiplegic migraine (FHM) and spinocerebellar ataxia type 6 (SCA6) are neurological disorders, with extensive clinical overlapping, caused by mutations in a gene that encodes a subunit of a neuronal calcium channel (CACNA1A), involved in signaling pathways and neurotransmitter release. EA-2 is characterized by recurrent attacks of gait and limb ataxia, sometimes accompanied by dysarthria, vertigo, nausea and headache. FHM is a rare autosomal dominant subtype of migraine with aura, characterized by migraine attacks associated with transient hemiparesis. SCA6 is mainly characterized by progressive limb and gait ataxia. In EA-2 families, three nonsense and one splice-site mutations were reported in the CACNA1A gene; seven different missense mutations were described in FHM; SCA6 is caused by a (CAG)_n expansion in the 3'-UTR or by a missense mutation within exon 6. We have (1) assessed CAG repeat size at the SCA6 locus; (2) performed linkage analysis with chromosome 19p markers D19S1150, D19S840 and D19S226 and (3) screened our families with episodic ataxia and/or migraine for mutations in the CACNA1A gene. We ascertained 19 patients from one three-generation and three two-generation families with dominantly inherited episodic ataxia associated with migraine, as well as one isolated case. Polymorphism detection was performed by PCR amplification, single strand conformational polymorphism (SSCP) analysis and sequencing. Expansions of the CAG repeat were not present in any of these patients. Linkage analysis in the only informative family showed positive lod scores to chromosome 19p, but none of the mutations previously reported were found in these four families. In conclusion, we found an episodic ataxia family linked to CACNA1A markers, indicating that at least one additional mutation in the CACNA1A gene is responsible for this phenotype. We are now screening the remaining exons.

Founder effect of the spinocerebellar ataxia type 10 mutation in the Mexican population. *T. Ashizawa¹, T. Matsuura¹, A. Rasmussen², R.P. Grewal², L. Zu², S.M. Pulst², M. Pandolfo², H. Sasaki², V. Volpini², T. Yamagata^{1,2}, K. Watase¹, D.L. Burgess¹, K. Inoue¹, P. Yescas², S. Nagamitsu¹, M.Y. Momoi², K. Tashiro², H.Y. Zoghbi¹, E. Alonso², D.L. Nelson¹.* 1) Baylor College of Medicine, Houston, TX; 2) Others: NJ, CA, Mexico, Canada, Japan, Spain.

Spinocerebellar ataxia type 10 (SCA10) is an autosomal dominant disorder, characterized by cerebellar ataxia and epilepsy that was initially identified in two Mexican families. We recently determined that the mutation is an unstable expansion of a pentanucleotide (ATTCT) repeat with a gain of ~4000 >repeat units in intron 9 of the E46 gene on chromosome 22q13.3. We screened 29 unrelated Mexican probands with dominantly inherited ataxias (ADCAs). Of these 29 probands, four showed the SCA10 mutation. In contrast, we found no SCA10 among 19 Caucasian-American, 41 French-Canadian, 17 Japanese and 12 Spanish families with unassigned ADCA. We also examined the haplotype consisting of the ATTCT repeat itself and two flanking polymorphic markers spanning 79 kb in unaffected (n=130) and SCA10 (n=6) Mexican chromosomes. All six SCA10 families shared a common haplotype of markers in this region. This haplotype is the second most common in the Mexican population and is frequently associated with the upper normal range of the ATTCT repeat allele in the Mexican population; these distributions are distinct from those found in Caucasian and Japanese populations. Analyses of the disease haplotype and linkage disequilibrium suggest a strong founder effect in SCA10.

A positional cloning strategy to identify the gene for an autosomal dominant juvenile ALS on chromosome 9q34.

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Amyotrophic lateral sclerosis (ALS) denotes a heterogeneous group of progressive neurological disorders with degeneration of motor neurons. Childhood onset forms of familial ALS are termed juvenile ALS. We mapped an autosomal dominant form of juvenile ALS (ALS4) to 9q34 and refined the ALS4 locus to a 500kb interval (D9S149 and D9S1198). Using the GeneMap'99 EST databases, we identified over 60 genes in the form of ESTs that localize within the critical interval. Screening of EST databases with each mapped EST sequence has identified many overlapping unmapped EST sequences and generated extensive EST sequence contigs for most of these genes. Database comparisons with these extended sequences identified several transcripts whose predicted function suggested them as ALS4 candidate genes. Expression analysis and fine scale mapping has been used to investigate and prioritize transcripts prior to mutation analysis. Extensive mutation analysis was undertaken with many candidate genes using the techniques of dideoxy fingerprinting and direct sequencing. This has allowed us to exclude the known genes SYT7, GRIN1, STXBPI, ATSV, RING3L, TFIIC63, RalGDS, C9orf9, Endoglin, AMY, Forssman, TFIIC90, along with several new transcripts. Several transcripts mapping to the ALS4 candidate interval remain unscreened. We will utilize the resources of the human genome project to ensure all transcripts in the candidate interval are considered. Genomic sequence from marker D9S1198 to D9S1830 has been archived as part of the Whitehead Institute/MIT effort to sequence the TSC1 cosmid contig. Database sequence for the remainder of the ALS4 candidate interval exists as several overlapping unordered BAC fragments (Sanger Centre). We will use gene prediction programs (Genscan and Grail) to interrogate the available genomic sequence data and as a validation tool for our existing transcripts. Predicted exons may be screened directly for mutations, be useful in completing partial transcripts and identifying new genes.

Mutation dependent aggregation of tau protein and its selective depletion from the soluble fraction in brain of FTDP-17 patients. *P. Heutink, M. Hillebrand, R. Willemsen, A. Hoogeveen, P. Rizzu.* Dept Clinical Genetics, Erasmus Univ, Rotterdam, Netherlands.

Mutations in the gene for the microtubule associated protein tau are associated with frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17). The term FTDP-17 covers a number of neurodegenerative syndromes with overlapping clinical and neuropathological features. Early behavioral changes, later cognitive and motor disturbances, and atrophy in frontal and temporal lobes on neuroimaging, are characteristic for this condition. Pathological hallmarks of FTDP-17 brains are insoluble filamentous aggregates of hyperphosphorylated protein tau, in neurons or in both neurons and glial cells, similar to those found in other neurodegenerative disorders such as Alzheimer's disease, Pick's disease, Progressive Supranuclear Palsy, Cortico Basal Degeneration and Amyotrophic Lateral Sclerosis and Dementia complex of Guam. We compared the presence of the P301L mutated tau protein with the normal 4 repeat isoform, in brain material of patients using polyclonal antibodies specific for the P301L point mutation and its normal counterpart. We determined the relative ratio of mutated versus normal tau protein in the sarkosyl soluble and insoluble protein fractions from several brain regions. Although mutated and normal tau proteins are both present in the sarkosyl insoluble deposits, quantitative analysis showed that mutated tau protein is the major component of the deposits. The overall ratio of 3R tau isoforms vs. 4R tau isoforms is unchanged but there is a dramatic depletion of mutant tau protein in the sarkosyl soluble fraction of frontal and temporal cortex. Furthermore, tau immunoreactive cleavage products stained more strongly with the P301L antibody suggesting that mutant protein is more susceptible to specific protease degradation. This is the first direct evidence using patient material that shows a selective aggregation of mutant tau protein resulting in sarkosyl insoluble deposits and the specific depletion of mutated tau protein in the soluble fraction. We are currently characterizing the specific protease degradation of the mutant tau protein in brain.

Exclusion of nine candidate genes for their involvement in X-linked FG syndrome (FGS1) in three families. A.

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FG syndrome (MIM 305450) is a rare X-linked mental retardation syndrome characterized by severe mental retardation, macrocephaly, facial dysmorphism and anomalies in the development of the distal part of the digestive tract often resulting in anal anteversion or imperforation. Linkage analysis performed on a panel of 10 families have demonstrated that a gene responsible for the disease (FGS1) is located in the Xq12-Xq21 region of the human X chromosome with a maximal lodscore of 3.39 ($Z_{\max}=3.39$ at $q=0.12$) for the DXS441 marker located in Xq13.3 [Briault et al., 1997]. More recently, another study on three FG families showed that a FG syndrome gene was located in the Xq12-q22.1 region [Graham et al., 1998]. Taking into account the recombinants observed in the two studies, we have deduced that the FGS1 gene should be located between AR (Xq12) and DXS72 (Xq21), assuming homogeneity between the families in the two studies. We have thus decided to follow a systematic candidate gene approach in the linkage interval in order to identify the FGS1 gene. A first set of nine genes has been studied : XNP, BPX, E25, SH3BGRL, GPR23, CDX4, KIF4, GA17 and TAFII31L. TAFII31L is a co-activator of the p53 protein associated with the TFIID complex which was previously mapped to chromosome 5. Genomic sequence analysis shows that TAFII31 is located in this region of the human X chromosome instead. These nine genes have been analysed for their potential involvement in FGS1 by sequencing, expression analysis and southern blotting and were all found to be normal in the studied FGS1 patients.

MeCP2 mutations in Rett syndrome: Genotype and Phenotype evaluation of 83 patients. *K.C. Hoffbuhr¹, J. Devaney², N. Siranni¹, B. LaFleur¹, C. Scacheri¹, J. Giron¹, J. Schuette³, J. Innis³, M. Marino², M. Philippart⁴, S. Naidu⁵, E.P. Hoffman¹.* 1) Research Center for Genetic Medicine, Childrens National Medical Center, Washington DC; 2) Transgenomics Inc., Gaithersburg, MD; 3) Division of Pediatric Genetics, University of Michigan, Ann Arbor, MI; 4) Mental Retardation Research Center, UCLA, Los Angeles, CA; 5) Neurogenetics Unit, Kennedy Krieger Institute, Johns Hopkins University, Baltimore, MD.

The MeCP2 gene was screened in 83 patients with classical and atypical Rett Syndrome. We identified causative mutations in 60% of patients (50/83). Nine recurring mutations were found accounting for 74% of all gene changes. We found 15 novel mutations: 2 missense mutations [P322L, K305R], 2 nonsense mutations [S204X, Q244X], a A9963G splice site mutation, 2 insertions, and 8 frameshift deletions, predominantly clustered in the C-terminal region of the protein. Mutations in the MeCP2 gene were identified in two males: a Klinefelters male with classical Rett syndrome (T158M), and a hemizygous male infant with an Xq27-28 inversion and a novel 32 bp frameshift deletion [1154(32del)].

To examine if clinical severity was associated with specific mutation classes, 42 patients were scored on 5 clinical parameters and assigned to five mutation categories according to type of mutation (missense, nonsense or deletion) and location of the mutation within the gene (methyl binding domain, transcriptional repression domain, or 3 of these regions). Preliminary analysis indicated that mutations in the amino-terminus were correlated with more severe phenotypes, specifically missense mutations within the methyl-binding domain of the protein or mutations resulting in the complete loss of the transcriptional repression domain. Quantitative X-inactivation studies of 25 patients revealed skewed X-inactivation (> 85% of one X active) in 4 patients, 3 with a high functioning Rett phenotype and one with a classical Rett presentation. These studies suggested that X-inactivation can be an important determinant of phenotype in Rett syndrome.

Investigation of the genetics and molecular pathology of Rett syndrome. A. McDonnell, M. Ennis, L. Torrie, E.F. MacDougall, A.M. Kerr, G.J.M. Brock, M.E.S. Bailey. Division of Molecular Genetics, I.B.L.S., University of Glasgow, Glasgow, U.K.

Rett Syndrome is a severe neurodevelopmental disorder that is inherited in an X-linked, genetically dominant fashion. The molecular genetics of Rett Syndrome has been investigated in a cohort of patients (n=40) from the UK. Initially, a candidate gene approach was taken, during which the structure of the GABA_A receptor q subunit gene, *GABRQ*, located within Xq28, was investigated. The expression patterns of this gene and two others located in the same gene cluster suggested them as candidates for Rett syndrome. We have determined the position and sequence of all the intron/exon boundaries in *GABRQ*, and have identified in unrelated individuals a set of single nucleotide polymorphisms (SNPs) that will be of use in association analyses.

Rett syndrome has in fact been shown to be caused by mutations in the methyl CpG binding protein 2 gene, *MECP2*. We have genotyped Rett families throughout most of this gene, which is now known to possess 4 exons. We have used intronic primers to sequence exons 2 and 3 completely and we have also sequenced both the coding portions of exon 4 and the newly defined exon 1, including approx. 300 bp of putative promoter sequence upstream from the likely transcription initiation site. We have found mutations in a significant proportion of our patients, including several novel mutations. We have also commenced the investigation of the long form of the 3'-UTR, using multiple sets of overlapping primers, in those patients in which no mutations were detected in other regions of the gene. We have initiated studies of the functional effects of point mutations in the methyl binding domain (MBD) of human MECP2 on binding of the domain to its natural genomic targets, identified through the use of an MECP2 MBD binding column. We have identified and characterised a number of novel, single copy genomic fragments that bind strongly to this column, as well as a range of multicopy repeats. The relative representation of different repeat classes in the library has been compared with their genomic distributions.

Clustering of disorders of peripheral myelin in the Vlax Roma: identification of three novel neuropathies. *L.V. Kalaydjieva¹, T. Rogers¹, R. Gooding¹, D. Gresham¹, D. Angelicheva¹, D. Chandler¹, I. Tournev², P.K. Thomas³*. 1) Center for Human Genetics, Edith Cowan Univ, Perth WA, WA, Australia; 2) Department of Neurology, Medical University, Sofia, Bulgaria; 3) Institute of Neurology, University College London, UK.

In our studies of the Vlax Roma (Gypsies), we have identified three novel autosomal recessive neuropathies caused by founder mutations that occur at high frequencies and often segregate independently in the same kindreds: Hereditary Motor and Sensory Neuropathy - Lom (HMSNL), the complex neurodevelopmental disorder Congenital Cataracts Facial Dysmorphism Neuropathy (CCFDN) syndrome and Hereditary Motor and Sensory Neuropathy - Russe (HMSNR). High gene frequencies and old mutation ages preclude homozygosity mapping despite consanguinity, but at the same time provide a valuable tool in the refined mapping of the disease genes.

HMSNL was recently shown to result from a truncating mutation in the N-myc downstream-regulated gene 1, previously suggested to play a role in growth arrest and cell differentiation and to have tumour-protective effects. CCFDN was mapped to 18qter and the characterisation of a candidate gene, encoding a highly conserved zinc finger protein, is currently in progress. HMSNR was mapped to 10q23, in close proximity to an excellent candidate, the Early Growth Response 2 (EGR2) gene, known to be important for PNS myelination. EGR2 was excluded by sequencing and SNP analysis, indicating that HMSNR is caused by a novel gene in the same region. Refined mapping of the gene interval is in progress. The neuropathological findings in the three novel neuropathies suggest that the responsible genes are involved in Schwann cell differentiation, myelination and the processes of Schwann cell/axonal signalling. Their identification will provide novel information on PNS neurobiology.

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Analysis of splice site mutations of the proteolipid protein (PLP) gene in Pelizaeus-Merzbacher disease. *G.M.*

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Mutations of the proteolipid protein (PLP) gene cause a wide spectrum of phenotypes from the relatively mild X-linked spastic paraplegia to the severe, connatal form of Pelizaeus-Merzbacher disease, which is only partially explained by the variety of genetic lesions of the PLP gene that are known to cause disease. Duplications (most common) and triplications, but also a complete gene deletion, null mutations, missense mutations, nonsense mutations and small deletions and insertions are all known to cause disease. The wide spectrum of severities and variety of mutations in PLP create a unique model for studying phenotype/genotype correlations. We have examined mutations that affect PLP mRNA splicing. The PLP gene not only codes for PLP mRNA, but also for DM20 mRNA, due to the use of an alternative splice site within PLP exon 3, resulting in an mRNA that lacks 105 bp. Missense mutations in this PLP-specific fragment are reported to cause a relatively mild disorder, X-linked spastic paraplegia, suggesting that mutations affecting PLP but not DM20 give a mild phenotype. We have examined PLP and DM20 mRNA from cultured skin fibroblasts of patients with the following mutations: T115K, which results in a better fit of the DM20 splice donor site with the consensus splice site; K150N, which, in addition to being a missense mutation in the PLP-specific region, results in a poor fit of the PLP splice donor site with the consensus splice site; and IVS3+2 and IVS3+4, which result in a poor fit of the PLP splice donor site with the consensus splice site. All four mutations severely affect levels of PLP mRNA, while the levels of DM20 mRNA are less affected, if at all. Like missense mutations in the PLP-specific region, the T115K mutation results in mild disease. However, patients with the other three splice site mutations have a more severe phenotype.

Lethal Autosomal Recessive Nephrotic Syndrome, Microcephaly, Cerebellar Hypoplasia: possible Galloway Mowat Syndrome. *R. HAMED¹, T. SRIVASTRA², R.E. GAROLA², M.J. DASOUKI²*. 1) DEPARTMENT OF PEDIATRICS, UNIVERSITY OF JORDAN, AMMAN, AMMAN, JORDAN; 2) CHILDREN'S MERCY HOSPITALS & CLINICS. KANSAS CITY. MO.

Early onset nephrotic syndrome and central nervous system anomalies are the main features of Galloway-Mowat syndrome, a rare autosomal recessive disorder. The renal histopathology varies from mild glomerular lesion to diffuse mesangial sclerosis. Whether these changes represent heterogeneity within the syndrome or different stages of disease progression is not known. We report two unrelated and consanguineous Palestinian/Jordanian families with 6 children [4 boys and 2 girls] afflicted with a lethal syndrome that consists of CNS abnormalities [microcephaly, hypertonia, seizures, cerebellar hypoplasia], failure to thrive and steroid resistant nephrotic syndrome. Death occurred by 3.5 years of age in all of them except in 1 girl who is still living but critically ill. Renal biopsies done in 3 children showed immature glomeruli, mild mesangial proliferation and focal segmental sclerosis. Immunofluorescence showed mesangial deposits of IgG, IgA, IgM and C3. Synaptopodin is an actin-associated protein localized to telencephalic dendrites in brain and podocytes in kidney. Its synthesis in the telencephalon has been shown by in situ hybridization, where synaptopodin mRNA was found in perikarya of the olfactory bulb, cerebral cortex, striatum, and hippocampus making it a candidate gene for this disorder. Using mouse antisynaptopodin, the renal expression was examined in available kidney biopsies and was found to be decreased but not absent. These results suggest that the predicted synaptopodin mutations (if present) are non-truncating. Mutation analysis for such mutations in the synaptopodin gene should help confirm our hypothesis.

Peripheral neuropathy with hypomyelination, chronic intestinal pseudo-obstruction and deafness related to a SOX10 mutation. *V. Pingault¹, A. Guiochon-Mantel², N. Bondurand¹, C. Faure³, C. Lacroix⁴, S. Lyonnet⁵, M. Goossens¹, P. Landrieu⁴.* 1) INSERM U468, Hosp Henri Mondor, Creteil, France; 2) Hormonologie et Biologie Moleculaire, Hop Bicetre, Le Kremlin-Bicetre, France; 3) Gastroenterologie Pediatrique, Hop R.Debre, Paris, France; 4) Neurologie Pediatrique/Neuropathologie, Hop Bicetre, Le Kremlin-Bicetre, France; 5) INSERM U393, Hop Necker, Paris, France.

We describe the case of a girl with an unusual congenital phenotype, combining peripheral nerve lesions with hypomyelination, chronic intestinal pseudoobstruction and deafness. She was found to have a de novo heterozygous frameshift mutation in the gene encoding SOX10, a member of the SRY-related family of transcription factors characterized by a high-mobility-group DNA binding domain. SOX10 mutations have been described in Waardenburg-Hirschsprung disease, which is characterized by intestinal aganglionosis, pigmentation disorders and deafness. The likely role of SOX10 in determining the fate of Schwann cells during early embryogenesis may explain the peripheral nervous system developmental disorder observed in this patient.

Hereditary Spastic Paraplegias in Italian families: Clinical and Molecular Investigations. *C. Patrono¹, F. Cricchi², D. Fortini², A. Tessa¹, G. Tozzi¹, F. Piemonte¹, R. Carrozzo¹, E. Bertini¹, G. Comanducci², C. Casali², F.M. Santorelli¹.* 1) Molecular Medicine, IRCCS-Bambino Gesù, Rome, RM, Italy; 2) Neurological Institute, La Sapienza University, Rome, RM, Italy.

Hereditary spastic paraplegias (HSP) are characterized by progressive weakness and spasticity of the lower limbs due to degeneration of corticospinal axons. Genetic heterogeneity is suggested by different modes of transmission (dominant, recessive, and X-linked) and clinical presentations (pure and complicated HSP). Recently the genes responsible for the autosomal recessive SPG7 and the dominant SPG4 form have been cloned. SPG7 encodes a mitochondrial AAA-metalloprotease (paraplegin), suggesting an altered OXPHOS mechanism for neurodegeneration in HSP. SPG4 encodes a still uncharacterized AAA-protein (spastin). We investigated 7 AD, 2 AR-HSP families and 9 apparently sporadic cases. Muscle biopsies were obtained in 10 patients for morphologic and biochemical studies. Molecular characterization included SPG4 and SPG7 genes. Both pure and complicated forms have been recognized in our series. Optic neuropathy, retinopathy, dementia, ataxia, and deafness were found in the complicated forms. MRI disclosed a thin corpus callosum in two recessive patients but linkage analysis ruled out involvement of the recessive locus on chromosome 15q (SPG11). In the biopsied muscles of HSP patients, we found reduced stain for COX in 1 case and SDH-hyperintense stain of mitochondria in 2. Biochemically, we found a significant reduction of Complex I activity in 4 patients when values were corrected for their total mitochondrial mass. While we did not detect SPG7 mutations, we identified three new SPG4 mutations. SPG4 patients did not show OXPHOS alterations in muscle biopsies. Our data reinforce the notion of the ample variability in the presentation of HSP and confirm the relative rarity of the SPG7-associated form. Defective OXPHOS mechanisms could play a role in the pathogenesis of the disease in a subgroup of patients. Supported by: Ricerca Finalizzata, Ministero della Sanit.

Molecular studies of childhood onset primary dystonias in Italy. *B. Garavaglia¹, F. Invernizzi¹, N. Nardocci², G. Zorzi², F. Girotti³, M. Zeviani¹.* 1) Div. Biochimica e Genetica,; 2) Div. Neuropsichiatria Infantile,; 3) Div. Neurologia I, Istituto Neurologico "C.Besta", Milano, Italy.

Primary dystonias are a clinically and genetically heterogeneous group of movement disorders with dystonia as a major symptom. Disease genes have been identified in three autosomal dominant forms (early-onset primary torsion dystonia, PTD, dopa-responsive dystonia, DRD, and myoclonus-dystonia syndrome, MD) and in one X-linked recessive form. A 3-bp GAG deletion at nt 946 in the DYT1 gene is the cause of most cases of familial PTD, especially among Ashkenazi Jews. The GTP cyclohydrolase 1 gene (DYT 5) is mutated in DRD. More recently, a missense mutation in the D2 dopamine receptor (DRD2) gene has been found in association with MD. However, since this mutation was present in several members of a single family, its pathogenicity is still controversial. A molecular analysis was performed on a large cohort of non-Jewish Italian patients. The 3-bp GAG heterozygous deletion was found in 5/28 unrelated patients with early onset PTD. Although family history was negative, the GAG deletion was found in all of the three fathers that underwent the genetic test, and in a sister of an affected male patient. In our study, the frequency of DYT 1 mutation is 18%, a figure similar to that of other reports on non-Ashkenazi Jewish patients. This result indicates the presence of high genetic heterogeneity in PTD. The DYT 5 gene was sequenced in six DRD patients from four unrelated families. We found three new heterozygous mutations in three families, while no mutation was found in the fourth. A frameshift mutation in exon 6 was found in one patient, a splice-site mutation in intron 5 was found in a father and a son, and a missense mutation in exon 5 was found in two sibs. Incomplete penetrance of the latter mutation was suggested by its detection in the asymptomatic mother. In the only MD patient so far examined we found a missense mutation in exon 6 of the DRD2 gene. This mutation changes a highly conserved Ser into a Cys residue. The second observation of a DRD2 gene mutation in MD syndrome strongly suggests an etiological relation between DRD2 gene defects and the specific MD phenotype.

IL1RAPL1 is Responsible for Mental Retardation in Patients with Complex Glycerol Kinase Deficiency who have Deletions Extending Telomeric from DAX1. *Y.-H. Zhang¹, K. Dipple¹, B.-L. Huang¹, L.L. McCabe^{1,2}, E.R.B. McCabe¹.* 1) Pediatrics; 2) Human Genetics, UCLA Sch Medicine, Los Angeles, CA.

IL1RAPL1 (interleukin-1 receptor accessory protein-like, gene 1) has recently been shown to be mutated in patients with X-linked mental retardation mapping to Xp22.1-p21.3 (Carrie et al, *Nature Genet* 23:25-31, 1999) and in one family with the contiguous gene syndrome, complex glycerol kinase deficiency (cGKD) (Jin et al, *Eur J Hum Genet* 8:87-94, 2000). Clinical experience has suggested that patients will have mental retardation (MR) if they have deletions extending from the GK gene into the DMD gene (presumably due to altered central nervous system expression of dystrophin) and/or involving a significant extension telomeric from DAX1 (responsible for adrenal hypoplasia congenita, AHC). The goal of our investigation was to examine cell lines from patients with cGKD whose clinical features we determined would be informative and would allow us to test the hypothesis: IL1RAPL1 is sufficient to explain the MR in patients with deletions extending telomeric from DAX1. Our results showed that patients with deletions involving DAX1 and GK, but not DMD: (1) had MR if IL1RAPL1 was deleted; or (2) did not have MR if IL1RAPL1 was intact. Deletions in DNA from patients with cGKD who exhibited MR and had normal IL1RAPL1 all involved the DMD gene. A very interesting family was the one we originally described with this syndrome (McCabe et al, *Biochem Biophys Res Commun* 78:1327-1333, 1977; Guggenheim et al, *Ann Neurol* 7:441-449, 1980) in whom the breakpoints were within the IL1RAPL1 and DMD genes, and therefore similar to the family described by Jin et al. We conclude that our data are consistent with the IL1RAPL1 gene being sufficient to explain the MR associated DAX1-GKD deletions. Some patients' deletions are quite large and the data do not exclude the presence of additional MR genes distal to IL1RAPL1. In summary, the data on IL1RAPL1 deletions in patients with cGKD provide a molecular genetic explanation for the MR observed in some patients, and will help us provide prognostic information to families of children with this contiguous gene syndrome.

Genetic analysis of Mexican families with Alzheimer's disease. *P. Yescas, J. Guerrero, A. Rasmussen, ME. Alonso.* Department of Genetics, Instituto Nacional de Neurologia y Neurocirugia Manuel Velasco Suarez, Mexico City, DF, Mexico.

Alzheimer's disease (AD) is the most common dementia of adulthood. The prevalence of the disease has not been determined in Mexico, but the analysis of the files of the National Institute of Neurology reported that 0.29% of all patients were diagnosed with AD. The majority of AD patients are sporadic cases, and only 5% have an autosomal dominant inheritance. In some of these familial cases, the disease is caused by mutations in the b-amyloid precursor protein, presenilin 1 (PS1) or presenilin 2 genes. The ϵ_4 allele of the Apolipoprotein E gene is considered a risk factor mainly for sporadic and late onset AD. In the present report we describe seven families with early onset autosomal dominant AD. Average age of onset in these families was 40 years (range 36 to 43 years). Two of the probands died after 7 and 10 years of disease respectively; the diagnosis was confirmed at autopsy. Apo E alleles were typified in all probands and only one individual was a 4/3 heterozygote, the rest being 3/3 homozygotes. Mutation analysis of the PS1 gene is being performed. Exons 5, 6, 7 and 8 have been amplified and digested with restriction enzymes, without finding any changes in the expected restriction pattern. This implies it will be necessary to sequence these exons and possibly the whole gene in order to rule out mutations in PS1 as the cause of the disease. Financial support by CONACYT ref 28664M.

Mutation analysis of the spastin gene in hereditary spastic paraplegia type 4---evidence of aberrant transcript splicing caused by mutations in noncanonical splice site sequences. *I.K. Svenson¹, A.E. Ashley-Koch¹, P.C. Gaskell¹, T.J. Riney¹, C. Warner², C.D. Farrell³, R.-M.N. Boustany¹, J.L. Haines⁴, M.A. Nance⁵, M.A. Pericak-Vance¹, D.A. Marchuk¹.* 1) Duke University Medical Center, Durham, NC; 2) Dent Neurologic Institute, Buffalo, NY; 3) Roswell Park Cancer Institute, Buffalo, NY; 4) Vanderbilt University Medical Center, Nashville, TN; 5) Park Nicollet Clinic, Minneapolis, MN.

Pure hereditary spastic paraplegia type 4 (SPG4) is the most common form of autosomal dominant hereditary spastic paraplegia, a neurodegenerative disease characterized primarily by hyperreflexia and progressive spasticity and weakness of the lower limbs. SPG4 was recently found to be caused by mutations in the gene encoding spastin, a member of the AAA family of ATPases (Hazan *et al.*, *Nature Genetics* 23:296, 1999). We have screened the spastin gene for mutations in the probands of 15 pure SPG families for which the lod score at SPG4-linked markers is three or greater, or in the case of smaller families is consistent with the theoretical maximum. By sequencing all 17 exons and flanking intronic sequence, we identified mutations in 11 of the families. In addition to nonsense and missense mutations and a single-nucleotide exonic insertion, we identified 7 splice site or potential splice site mutations. Interestingly, 5 of these are located in noncanonical splice site sequences, for two of which patient RNA was available. RT-PCR analysis in these two cases revealed aberrant splicing---deletion of an entire exon in one case and creation of a novel splice site resulting in a 4bp deletion from an exon in the other case. In the former case, the aberrant transcript is much less abundant than the wild type, suggesting that it is less stable or is expressed at a lower level, or that the mutation is not fully penetrant. We are currently investigating this latter possibility by cloning and expressing the mutant and wild-type alleles separately in COS cells, with a view toward identifying the mechanisms by which these mutations cause SPG.

Expanded polyglutamine stretches associated with CAG repeat diseases interact with TAF_{II}130, interfering with CREB-dependent transcription. *T. Shimohata*¹, *T. Nakajima*², *M. Yamada*³, *C. Uchida*⁴, *O. Onodera*¹, *S. Naruse*¹, *T. Sato*¹, *T. Kimura*¹, *K. Nozaki*¹, *Y. Sano*¹, *A. Sato*¹, *M. Oyake*¹, *N. Tanese*⁵, *H. Takahashi*³, *S. Tsuji*¹. 1) Departments of Neurology, Brain Research Institute, Niigata University, Niigata, Japan; 2) Institute of Applied Biochemistry, TARA center, University of Tsukuba, Ibaraki, and PRESTO JST, Institute of Medical Science, St. Marianna University of Medicine, Kawasaki, Japan; 3) Departments of Pathology, Brain Research Institute, Niigata University, Niigata, Japan; 4) Department of Biochemistry, Hamamatsu University School of Medicine, Shizuoka, Japan; 5) Department of Microbiology and Kaplan Comprehensive Cancer Center, New York University Medical Center.

At least eight inherited neurodegenerative diseases have been identified to be caused by expanded CAG repeats encoding polyglutamine (polyQ) stretches. Given that the nuclear translocation of mutant proteins containing expanded polyQ stretches is a prerequisite for their cytotoxicity, we hypothesized that nuclear proteins that interact with the mutant proteins, in particular, those that bind to the expanded polyQ stretches, may be involved in the pathogenetic mechanisms underlying neurodegeneration. We found that expanded polyQ stretches bind to a TATA-binding protein (TBP)-associated factor (TAF_{II}130), a coactivator involved in cAMP-responsive element-binding protein (CREB)-dependent transcriptional activation. The binding of the expanded polyQ stretches to TAF_{II}130 was confirmed by four independent methods, including the yeast two-hybrid, Far Western, coprecipitation and colocalization assays. We furthermore found that expanded polyQ stretches strongly suppressed CREB-dependent transcriptional activation, which was restored by coexpression of TAF_{II}130. Expanded polyQ-induced cell death was also substantially rescued by coexpression of TAF_{II}130. We propose that interference with transcriptional activation due to the binding of the expanded polyQ stretches to TAF_{II}130 is involved in the pathogenetic mechanisms underlying CAG repeat diseases.

Neuronal expression of the fukutin gene. *J. Sasaki*^{1,2,3}, *K. Ishikawa*⁴, *K. Kobayashi*^{1,2}, *E. Kondo-Iida*², *Y. Sakakihara*³, *M. Fukayama*³, *H. Mizusawa*⁴, *S. Takashima*⁵, *Y. Nakamura*², *T. Toda*^{1,2}. 1) Division of Clinical Genetics, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; 2) Human Genome Center, Institute of Medical Science, Minato-ku, Tokyo, Japan; 3) Departments of Pediatrics and Pathology, University of Tokyo, Bunkyo-ku, Tokyo, Japan; 4) Department of Neurology, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan; 5) National Institute of Neuroscience, NCNP, Kodaira, Tokyo, Japan.

Fukuyama-type congenital muscular dystrophy (FCMD), a relatively common autosomal recessive disorder in the Japanese population, is characterized by severe congenital muscular dystrophy in combination with cortical dysgenesis (micropolygyria). We identified on chromosome 9q31 the gene responsible for FCMD, which encodes a novel 461-amino-acid protein suggested to be an extracellular protein. Pathological study on the brain of the FCMD fetuses had revealed that the glia-limitans and basement-membrane complex had frequent breaks. Thus, a structural alteration of the basal lamina appears to play a key role in the pathophysiology of FCMD. To investigate a role of fukutin in anomaly formation, we examined the fukutin gene expression in the human brains. Northern blot and RT-PCR analyses revealed that the fukutin gene was expressed relatively equally in various fetal tissues and various adult brain regions. Its expression was much reduced in FCMD brains. Tissue in situ hybridization analysis demonstrated that the fukutin mRNA was expressed in the migrating neurons including Cajal-Retzius cells and adult cortical neurons as well as the hippocampal pyramidal cells and cerebellar Purkinje cells. However, the expression was not observed in the glia-limitans, subpial astrocytes that produce the basement membrane, and other glial cells. In FCMD brains, neurons in the region that has no dysplasia showed a fair expression, whereas the transcripts were nearly undetectable in the overmigrated abnormal region. These suggest that 1) fukutin may be related not only to the basement membrane but to the neuronal migration itself and 2) the difference of mRNA amount among neurons in the early developmental stage may partially discriminate the normal and abnormal area.

A mutation in the Rett Syndrome gene, MECP2, causes X-linked mental retardation and progressive spasticity in males. A. Renieri¹, I. Meloni¹, M. Bruttini¹, I. Longo¹, F. Mari¹, K. Denvriendt², J.-P. Fryns², D. Toniolo³. 1) Genetica Medica, University of Siena, Italy; 2) Genetics Department, University of Leuven, Belgium; 3) Istituto di genetica Biochimica ed evoluzionistica, CNR (IGBE-CNR), Pavia, Italy.

Heterozygous mutations in the X-linked MECP2 gene cause Rett syndrome, a severe neurodevelopmental disorder of young females. No males with MECP2 mutations are reported to survive beyond 1 year of age suggesting the hypothesis that mutations in MECP2 are male lethal. Here we report a three generation family in which two affected males showed severe mental retardation and progressive spasticity (1). Two obligate carrier females showed either normal or borderline intelligence simulating a X-linked recessive trait. The two males and the two obligate carrier females presented a mutation in the MECP2 gene leading to substitution of glutamine (CAG) 406 with a stop codon (TAG). The X inactivation assay, while not informative in one female, showed completely balanced X inactivation the other. Since a notable variation between tissues is reported, the presence of a completely skewed X-inactivation in relevant tissues, like brain, cannot be excluded. An alternative hypothesis is that Rett syndrome is a digenic disease which develops when a de novo mutation in MECP2 gene occurs in the presence of a mutation in another gene. Following this digenic model MECP2 mutation alone produces a recessive phenotype, while mutation in the second gene alone may not produce phenotypic effect at all. A combination of both mutations in males could produce a more severe phenotype possibly lethal. We suggest that mutation in MECP2 gene may account for a subset of apparently recessive X-linked mental retardation and that mutation analysis of MECP2 gene should be performed also in mentally retarded males with progressive encephalopathy. Extensive analysis of MECP2 gene in such cases will give an idea about the percentage of X-linked mental retardation due to a MECP2 mutation. 1) Claes S, et al. X-linked severe mental retardation and a progressive neurological disorder in a Belgian family: clinical and genetic studies. Clin Genet 52:155-61, 1997.

PROSAP2: A candidate gene for neurological abnormalities in the 22q13 deletion syndrome. *H.L. Wilson, A.C.C. Wong, W.Y. Tse, H.E. McDermid.* Biological Sciences, University of Alberta, Edmonton, Alberta, Canada.

Patients with the 22q13 deletion syndrome show hypotonia, moderate to severe mental retardation, delay of expressive speech, normal to accelerated growth and mild dysmorphic features. A patient with mental retardation and delay of expressive speech was found to have a 130KB microdeletion of this region, defining the critical region for a subset of the neurological abnormalities associated with this syndrome. Three genes have been identified in the microdeletion region: 1) ACR (acrosin), a serine protease found in the acrosome of sperm heads, 2) RABL2B, involved in vesicular trafficking, with a near identical copy on chromosome 2, and 3) PROSAP2, the homologue of ProSAP2, recently identified in the rat (Boeckers, et al., 1999). ProSAP2 localizes to the post-synaptic density region of neurons, and shows a brain specific expression with a single transcript in rats. In humans, however, we have found multiple transcripts of PROSAP2 in all tissues tested by Northern blot analysis. One transcript, however, is predominantly expressed in the brain. This transcript is expressed in all brain tissues tested, but with low levels in the spinal cord and medulla. PROSAP2 is a member of a newly described family of proteins that are involved in connecting ion channels and receptors in the post-synaptic membrane to the cytoskeleton and to signal transduction pathways. As such, PROSAP2 is a promising candidate gene for the involvement in the neurological abnormalities of the 22q13 deletion syndrome. Since the critical region has been defined by a single patient, we are determining the extent of the deletions in more than 30 patients to confirm that PROSAP2 is deleted in all cases, and to compare the deletion location to the phenotype. To date, this gene is deleted in all patients tested, providing support for PROSAP2 as a candidate gene for the neurological abnormalities seen with this syndrome.

Frataxin deficiency enhances apoptosis in P19 cells neuronal differentiation. *M. Santos, M. Pandolfo.* Centre de Recherche, CH Université de Montréal, Montreal, Quebec, Canada.

Deficiency of the mitochondrial matrix protein frataxin causes Friedreich ataxia. Frataxin has an unknown function, thought to be related to mitochondrial iron metabolism and free radical production. In Friedreich ataxia, loss of dorsal root ganglia neurons is an early, probably developmental process. In addition, frataxin knock-out mice die during embryonic life, suggesting that frataxin is necessary for normal development. We genetically manipulated P19 mouse embryonic carcinoma cells to generate frataxin deficient clones by stable transformation with an antisense construct. Stable transfectants have 10-20% of the non-transfected cells level of frataxin. We then differentiated these cells into neurons by exposure to retinoic acid (RA) and into cardiomyocytes by exposure to DMSO. We evaluated cell survival and capacity to differentiate. When neuronal differentiation of P19 cells is induced by retinoic acid, cells respond with a growth arrest, some go into apoptosis, most differentiate to eventually express neuronal markers, including the neurotrophin receptor TrkC. Frataxin expression is markedly induced during this process. Frataxin deficient cells generate only very few differentiated cells expressing neuronal markers. Most of them go into apoptosis 12-72 hours after induction of differentiation with RA. Surprisingly, frataxin deficiency does not affect cell survival in DMSO-induced differentiation into cardiomyocytes. This simple in vitro model of neuronal differentiation shows that frataxin deficiency renders cells apoptosis-prone when exposed to appropriate stimuli, as RA. Vulnerability to apoptosis during development may explain the neuronal loss in dorsal root ganglia observed in Friedreich ataxia.

Nuclear accumulation of caspase-cleaved huntingtin in stressed human lymphoblasts: Signaling from mitochondria to nucleus via caspase-3 in HD pathology. *S. Sutcliffe*^{1,2}, *X. Luo*¹, *J. Cheah*¹, *E. Nagata*¹, *R. Margolis*³, *A.H. Sharp*³, *C.A. Ross*^{1,3}, *S.H. Snyder*^{1,3}, *A. Sawa*^{1,3}. 1) Neuroscience, Johns Hopkins University, Baltimore, MD; 2) Epidemiology, Johns Hopkins University, Baltimore, MD; 3) Psychiatry, Johns Hopkins University, Baltimore, MD.

Huntingtons disease (HD) is a hereditary neurodegenerative condition caused by a characteristic mutation in the huntingtin gene (htt). The nucleus and mitochondria have both been implicated in HD pathology. Various lines of evidence suggest the importance of nuclear localized Htt or Htt fragments in HD pathology. However, the mechanism and role of htt cleavage and localization to the nucleus remains to be elucidated. We have previously demonstrated that mitochondrial abnormalities in stressed HD lymphoblasts lead to caspase-3 overactivation. In in vitro recombinant studies of htt, caspase-3 was found to be capable of cleaving htt. In this study, we have attempted to evaluate htt cleavage in stressed HD and control lymphoblasts. By western blot analysis, we observed both caspase-dependent cleavage, blocked by caspase inhibitors, and caspase-independent cleavage of htt. We further estimated the sites of caspase-3 cleavage in htt, using various antibodies against epitopes near putative caspase-3 cleavage sites. By subcellular fractionation and immunofluorescent staining, we confirmed the enrichment of caspase-dependent cleavage products in the nucleus. We are now investigating the kinetics of caspase-dependent cleavage and possible sorting mechanisms to the nucleus. We propose that there is a pathological signaling cascade from the mitochondria to the nucleus contributing to HD progression.

Evidence for Genetic Heterogeneity in Rothmund-Thomson Syndrome. *L.L. Wang¹, M.L. Levy¹, R.A. Lewis¹, A. Gannavarapu¹, D.W. Stockton¹, L.M. Burks¹, D. Lev², C.M. Cunniff³, S.E. Plon¹.* 1) Baylor College of Medicine, Houston, TX; 2) Institute of Medical Genetics, Wolfson Medical Center, Holon, Israel; 3) U. of Arizona HSC, Tucson, AZ.

Rothmund-Thomson Syndrome (RTS) is a rare autosomal recessive genodermatosis characterized by poikiloderma in infancy, small stature, skeletal abnormalities, juvenile cataracts, and predisposition to osteosarcoma (OS). Mutations in *RecQL4*, which maps to 8q24.3 and encodes a RecQ DNA helicase, have been identified in a subset of patients with RTS by a candidate gene approach. The number of genes responsible for RTS is unknown since neither linkage studies nor cellular complementation analyses have been reported. We obtained DNA samples from a contemporary cohort of 28 patients to define the underlying genetic defect(s), clinical profile, and management of patients with RTS. We screened 13 probands for the four previously described mutations. One Hispanic subject with RTS and OS was homozygous for a nonsense mutation (Q757X) in the helicase domain of *RecQL4*. Two other Hispanic patients were heterozygous for this mutation. No probands carried the other reported mutations. A Mexican subject from a consanguineous family was homozygous for a missense alteration in a semi-conserved amino acid (P793S) of *RecQL4*, that appears to be a frequent polymorphism in the Hispanic population (3/31 Hispanic control subjects are heterozygous). Although RTS is typically autosomal recessive, two mother-son pairs have previously been described in the literature. One non-consanguineous family in our study had four affecteds in two successive generations suggestive of X-linked inheritance (2 males more severely affected than 2 females). We performed automated genotyping of markers along the X-chromosome and distal chromosome 8q. The sibling pairs were discordant for markers on chromosome 8q, but all four affecteds shared markers on chromosome Xp (DXS8051 to DXS993). Our data confirm that mutations in *RecQL4* are found in some patients with RTS and suggest that other genetic loci (possibly other helicases) may also be implicated.

Mutations spanning lamin A/C result in Emery-Dreifuss muscular dystrophy and adult onset isolated cardiomyopathy. C.A. Brown, C.J. Hartmann, R.W. Lanning, K.Q. McKinney, A.R. Salvino. Dept Pediatrics, Carolinas Medical Center, Charlotte, NC.

Emery-Dreifuss muscular dystrophy (EDMD) is characterized by early onset contractures of the elbows, Achilles tendons and spine, slowly progressive muscle wasting and weakness, and cardiomyopathy associated with conduction defects. The X-linked form is caused by mutations in the emerin gene mapped to Xq28, while mutations in the *LMNA* gene mapped to 1q21.2-21.3 are detected in some patients with autosomal forms of EDMD. *LMNA* mutations have also been found in patients with isolated dilated cardiomyopathy with conduction defects, limb girdle muscular dystrophy type 1B, and partial lipodystrophy. The *LMNA* gene encodes lamins A and C generated by use of an alternative splice site in exon 10. We used direct DNA sequencing to analyze exons 1-12 of the *LMNA* gene from 32 unrelated patients, 15 male and 17 female, clinically diagnosed with EDMD, and found not to have emerin gene mutations. *LMNA* mutations were found in 47% (15/32) of the patients. Of the mutations, 12 were missense, 2 were small in-frame deletions, and one altered a conserved donor splice site residue. The mutations were spread between exons 1 and 10, common to both lamin A and C. The majority of mutations introduce a proline residue or alter protein charge. Eight of the cases were familial, 2 confirmed to be *de novo* by molecular analysis, and 5 reported to be isolated cases based on negative family history. Intrafamilial phenotype variability was observed among patients harboring certain mutations within the central rod domain of lamin A/C, with clinical features ranging from childhood onset proximal muscle weakness and contractures to isolated adult onset cardiomyopathy. In conclusion, a significant percentage of patients with clinical features of EDMD were found to have *LMNA* gene mutations. Screening family members of index cases is important due to the variable phenotype associated with lamin A/C mutations and the life threatening complications of cardiac arrhythmias.

Absence of mutations in human ubiquitin fusion-degradation protein gene in tetralogy of Fallot. *M.Y. Chung¹, J.H. Lu^{2,3}, Y.Y. Weng¹, B. Hwang^{2,3}.* 1) Medical Research and Education, Veterans General Hospital-TPE, Taipei, Taiwan; 2) Pediatrics, VGH-Taipei; 3) Department of Medicine, National Yang-Ming University, Taipei.

Congenital defects in human chromosome 22q11 deletion syndromes are associated with the 3rd and 4th pharyngeal pouch during fetal development. In the cardiovascular system, these disorders are usually apparent as conotruncal heart defects and aortic arch anomalies. UFD1L, a gene which is down-regulated in dHAND deficient mice, expressed in mouse embryo at the branchial arch, and mapped to human chromosome 22q11, has recently been strongly suspected to be responsible for the phenotypes expressed in 22q11 deletion syndromes. Its putative causal role in relevant congenital cardiovascular malformations was studied by gene dosage analysis, mutation screening and sequence analyses. Sixty cases of tetralogy of Fallot with no detectable chromosome deletion at 22q11 or 10p13 were examined, including 51 cases of simple tetralogy of Fallot, and 9 cases of tetralogy of Fallot with pulmonary atresia. None of these patients revealed deletion limited to a portion of the UFD1L gene. Although mobility shift was found by heteroduplex analysis in 24 cases at exon 4 and flanking sequences, further sequence analysis demonstrated only two silent nucleotide variations and a single nucleotide polymorphism in intron 4. Our data suggest that, although the UFD1L gene is mapped to 22q11 and is expressed during early murine embryologic development at both cardiac and cranial neural crests, it is not responsible for the majority of tetralogy of Fallot in humans.

A New Genetic Locus for Thoracic Aortic Aneurysm/Dissection; Insight into the Molecular Pathology of the Defective Gene. *D. Guo¹, S. Kuang¹, S.N. Hasham¹, D. Abuelo², D.M. Milewicz¹*. 1) Internal Medicine, UT Medical School at Houston, Houston, TX; 2) Rhode Island Hospital, Providence, RI.

Thoracic aortic aneurysms and dissections (TAAs) are the major diseases that affect the aorta. Mutations in FBN1 result in Marfan syndrome (MFS), an autosomal dominant condition associated with TAAs. FBN1 mutations produce mutant fibrillin-1 (fib-1), which disrupts the polymerization of fib-1 into microfibrils. Ten families with autosomal dominant inheritance of TAAs in the absence of other features of the MFS were identified. Linkage analysis using markers within and surrounding FBN1 do not show linkage between FBN1 and the TAA phenotype. Genome wide screen and locus-specific linkage studies identified a genetic locus that demonstrated linkage to the phenotype. In one family the phenotype was not linked to this locus. Dermal fibroblasts explanted from affected individuals in 4 of these TAAs families were used for metabolic labeling studies of fib-1. All 4 cell strains produced normal amounts of fib-1, and secreted and processed the protein normally, but failed to assemble fib-1 into microfibrils. These data suggest that the defective gene responsible for TAAs may interact directly with fib-1 and disrupt microfibril formation, and therefore have a similar molecular pathology as FBN1 mutations.

Molecular Dissection of Velo-Cardio-Facial Syndrome Using Mouse Models. *S. Merscher¹, R. Russell¹, B. Funke¹, S. Factor¹, H. Xu¹, M. Lopez¹, A. Puech¹, B. St. Jore¹, H. Sirotkin¹, J. Heyer¹, P. Scambler², T. Wynshaw-Boris³, B. Morrow¹, A. Skoultschi¹, R. Kucherlapati¹.* 1) Molecular Genetics, Albert Einstein College of Med, Bronx, NY; 2) Institute of Child Health, London, England; 3) University of California, San Diego, La Jolla, CA 92093.

Velo-cardio-facial syndrome (VCFS) and DiGeorge syndrome (DGS) are autosomal dominant disorders that are characterized by a number of developmental abnormalities including conotruncal defects, facial dysmorphology, pharyngeal insufficiency and a plethora of other phenotypes. Most VCFS/DGS patients are hemizygous for a 3 Mb region of 22q11. A subset of patients has a 1.5 Mb nested deletion and the phenotypic spectrum of these patients is indistinguishable from those that have the 3 Mb deletion. Although the 22q11 region was mapped and sequenced, leading to the identification of genes, no single gene or a set of genes has emerged as the leading cause of the major phenotypes seen in VCFS/DGS patients. To facilitate the molecular dissection of the VCFS/DGS phenotypes, we generated a series of mice that carry deletions and duplications of parts of mouse chromosome 16 that correspond to human 22q11. In one case, we generated a deletion as well as a duplication of a 550 kb region that spans the interval between the genes *Idd-Arvcf*. Mice homozygous for this deletion do not survive embryogenesis, but the hemizygotes are normal. In the second case, we generated a deletion of nearly 1.5 Mb that spans the *Idd-Hira* region. Mice homozygous for this deletion also die during embryogenesis. Mice hemizygous for the deletion survive through embryogenesis but a high proportion of them die soon after birth. Examination of 18.5 dpc hemizygous embryos reveals that a large proportion of them have abnormalities of the outflow vessels from the heart such as interrupted aortic arch, an abnormal origin of the right subclavian artery (RSA) and an elongated brachiocephalic artery. These defects are similar to those seen in VCFS/DGS patients. Mice with this deletion are being mated with *Idd-Arvcf* duplication mice and BAC transgenics covering the region from *Arvcf-Hira* to define the region that harbors the gene(s) responsible for the heart defects.

Sarcoglycan is an intrinsic mediator of cardiomyopathy. *A.A. Hack¹, A. Heydemann², E.M. McNally².* 1) Dept Molecular Gen & Cell Biol, Univ Chicago, Chicago, IL; 2) Depts Medicine and Human Genetics, Univ Chicago, Chicago, IL.

In humans, mutations in the sarcoglycan genes have been associated with limb girdle muscular dystrophy and cardiomyopathy. In cardiac and skeletal muscle, the membrane bound sarcoglycan complex has both mechanical and signaling functions. In cardiac and skeletal muscle, the sarcoglycan complex includes at least four sarcoglycans, α , β , γ and δ . In contrast, smooth muscle has a more restricted sarcoglycan complex that is composed of β -, δ - and ϵ -sarcoglycan (Straub et al. 1999). Mice lacking δ -sarcoglycan were generated through gene targeting. Mice lacking δ -sarcoglycan develop cardiomyopathy and muscular dystrophy and die prematurely. In these δ -sarcoglycan deficient mice, the sarcoglycan complex is disrupted in smooth, cardiac and skeletal muscle raising the possibility that smooth muscle disruption of the sarcoglycan complex leads to cardiac degeneration and cardiomyopathy. To evaluate this question, we compared mice lacking γ -sarcoglycan to mice lacking δ -sarcoglycan since γ -sarcoglycan is not normally expressed in cardiac vascular smooth muscle. Mice lacking γ -sarcoglycan develop an identical cardiomyopathy to mice lacking δ -sarcoglycan with focal areas of degeneration, microscopic scarring, increased interstitial fibrosis and myocellular hypertrophy. Survival is similar in both γ - and δ -sarcoglycan deficient mice with 50% and 59% survival at 24 weeks for γ - and δ -sarcoglycan deficient mice, respectively. Importantly, the smooth muscle vascular sarcoglycan complex is not disrupted in mice lacking γ -sarcoglycan since we find normal expression of δ -sarcoglycan in the vascular smooth muscle of γ -sarcoglycan deficient mice. Therefore, smooth muscle disruption of vascular smooth muscle sarcoglycan is not required for cardiomyopathy. Additionally, perivascular fibrosis and vascular smooth muscle changes may occur as a secondary consequence in many intrinsic cardiomyopathic syndromes including those mediated by genetic mutations.

Defects in Laminin-a2 expression are associated with cardiomyopathy in murine models for Congenital Muscular Dystrophy. *G.K. Mack¹, S.M. Rash¹, G.A. Pantely², J. Pang¹, D.J. Sahn¹, D.M. Pillers^{1,3}.* 1) Department of Pediatrics; 2) Division of Cardiology, Department of Medicine; 3) Department of Molecular and Medical Genetics, Oregon Health Sciences University, Portland, OR.

Introduction: Muscular dystrophy (MD) affects a variety of organs including the neuromuscular, retinal, and cardiovascular systems. Congenital muscular dystrophy (CMD), is an autosomal recessive disorder that presents early in life with muscle weakness, is one group of MD with an associated cardiomyopathy. Laminin consists of three subunits, α , β , and γ . Laminin-2 contains isoform laminin- α 2. Mutations in the laminin- α 2 (LAMA-2) gene lead to a reduction or absence of laminin- α 2 in human patients and murine models. We hypothesized that a murine model of Laminin-2 deficiency would demonstrate a phenotype of hypertrophic cardiomyopathy compared with age and weight matched controls. **Methods:** 21 adult mice [8 wild-type C57BL6 controls, 8 *dy*, and 5 *dy2J*] ages 4 and 8 months were imaged by a 15MHz transducer for M-modes under 1% isoflurane. Triplicated measurements of left ventricular (LV) mass were calculated using a standardized formula corrected for heart muscle density and adjusted for body surface area (BSA) using Meeh's formula. The mean measurements were analyzed by a two-tailed Student's t-test assuming unequal variances. **Results:** Mean LV mass was 71.2mg for controls (avg wt 25.8g) and 90.2mg for *dy* (avg wt 16.3g), ($p=0.19$) and corrected LV mass was 0.63mg for controls and 1.03 mg/cm² for 4 mo *dy*, ($p<0.0002$). Mean LV mass was 57.2 for controls (avg wt 27.6g) and 111.8mg for *dy2J* (avg wt 47.3g), ($p<0.0003$) and corrected LV mass was 0.51 for controls and 0.69 mg/cm² for 8 mo *dy2J*, ($p<0.009$). **Conclusion:** Age matched mice with laminin- α 2 deficiency had significantly larger cardiac mass than controls. Alterations in Domain VI, corresponding to the amino-terminal globule of laminin- α 2, may affect the assembly of laminin and thus alter the structure and function of the basement membrane. We speculate that such a disruption is responsible for the cardiac phenotype in the murine models. Further studies are required to assess serial changes in cardiac mass, function, and expression of laminin- α 2 with age.

Sequencing of the beta myosin heavy chain gene in the patients with hypertrophic cardiomyopathy. M.V.

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Hypertrophic cardiomyopathy (HCM) is mainly genetically caused disease with high degree of allelic and locus heterogeneity, but almost all major genes code the sarcomeric contractile proteins. One of the most frequent mutated genes is beta-myosin heavy chain gene, which is responsible for approximately 50% of familial cases. To investigate genetic basis of HCM in the population of Russia, we have formed the sample of 24 individuals with HCM collected in Tomsk region. At the first stage of investigation of beta myosin heavy chain gene, the sequencing of exons 8, 9, 13, 14, 15, 19 has been performed (using automated ABI PRISM analyzer), including some parts of introns. We have not found any missense mutations in these exons comparing with the published sequence (Liew et al. 1990). However, it should be noted that all the samples have some differences with the published sequence in homozygous state, suggesting that there are some mistakes in the reference sequence. Most of these differences are located in introns 12 and 13 (single nucleotide insertions and deletions), and one difference is in the last position of exon 8, changing the last codon for F from TTT to TTC. Lacking of the missense mutations in these sequenced MYH7 exons in our sample confirms the great genetic heterogeneity of the disease. At the same time, we can not exclude the role of this gene as cause of HCM in this sample until other exons coding the head and head-rod regions of beta-myosin will be sequenced. Thus, future studies are needed for revealing genetic basis of HCM in Russian population.

A *de novo* translocation, t(8;14) (q22.3;q13), associated with Klippel-Trénaunay Syndrome (KTS). A.A. Timur¹, D.J. Driscoll², Q. Wang¹. 1) Center for Molecular Genetics, Cleveland Clinic Foundation, Cleveland, OH; 2) Section of Pediatric Cardiology, Mayo Clinic, Rochester, MN.

Klippel-Trénaunay syndrome (KTS) is a congenital vascular disorder comprised of capillary, venous, lymphatic malformations, and tissue hypertrophy. Recent studies in our laboratory suggest that approximately 50% of the KTS patients have other family members affected with various vascular disorders. Moreover, our laboratory has identified a balanced translocation in a KTS patient: t(8;14) (q22.3; q13). The parents and sister of the patient are not affected with KTS and they do not have the translocation, indicating that the translocation t(8;14) arose *de novo*. These findings suggest that genetic factors contribute to KTS. In this study, we aim to identify and characterize the gene involved in the pathogenesis of KTS. Finding and characterizing the KTS gene will uncover the molecular mechanisms underlying vascular morphogenesis. Our strategy is positional cloning of 8q22.3 and 14q13 breakpoints. Fluorescence *in situ* hybridization (FISH) and somatic cell hybrid analyses have been used to localize both translocation breakpoints. FISH experiments were carried out by using several YAC clones selected from the 8q22.3 and 14q13 breakpoint regions. We were able to localize chromosome 8q22.3 and 14q13 breakpoints between two YAC clones in a less than 5 cM region. We obtained five somatic cell hybrid clones containing derivative chromosome 14. These clones were screened by PCR for the presence of several markers located within the breakpoint regions. Our somatic cell hybrid analysis results also narrowed down both breakpoints in a less than 5 cM region.

Achromatopsia on chromosome 8q21 (ACHM3) is caused by mutations in the CNGB3 gene encoding the b-subunit of the cone photoreceptor cGMP gated channel. *S. Kohl¹, B. Baumann¹, M. Broghammer¹, H. Jaegle¹, P. Sieving², U. Kellner³, R. Spegal⁴, M. Anastasi⁵, E. Zrenner¹, L.T. Sharpe¹, B. Wissinger¹.* 1) University Eye Hospital, Tuebingen, Germany; 2) Kellogg Eye Center, University of Michigan, Ann Arbor, MI; 3) University Eye Hospital Benjamin Franklin, Berlin-Steglitz, Germany; 4) Micronesia Human Resource Development Center, Kolonia, Pohnpei State, Federated States of Micronesia; 5) Clinica Oculistica, Palermo, Italy.

We have shown that mutations in the CNGA3 gene encoding the a-subunit of the cone photoreceptor cGMP gated channel on chromosome 2q11 are responsible for achromatopsia, an autosomal recessive disorder characterized by total colorblindness, photophobia, reduced visual acuity and nystagmus. Another achromatopsia locus ACHM3 has been mapped to chromosome 8q21 by linkage analysis. Using achromatopsia families with exclusion of CNGA3 mutations, we confirmed linkage on chromosome 8q21 and refined the ACHM3 locus to a 3.7cM region between markers D8S1838 and D8S273. We established a YAC contig covering nearly the entire ACHM3 interval and isolated STS by means of Alu-vectorette PCR. Two STS matched to a HTGS sequence containing sequences homologous to the murine *cng6* gene encoding the putative b-subunit of the cone photoreceptor cGMP gated channel. We cloned the human cDNA homologue, CNGB3, corresponding to a 4.4kb transcript detected predominantly in the retina by means of RT-PCR and RACE. The human CNGB3 gene is encoded by 18 exons distributed over 200kb of genomic sequence. Analysis of the CNGB3 gene in linked achromatopsia families revealed 6 different mutations including a missense mutation (Ser435Phe), two stop codon mutations (Arg203stop, Glu336stop), a 1bp and a 8bp deletion (Thr383fs, Pro273fs), and a putative splice site mutation of intron 13. The Ser435Phe mutation was identified homozygously in a family originating from the Pingelap islander population. The Thr383fs mutation was identified recurrently and constituted 11 out of 22 disease chromosomes in our patient sample. Our data show that mutations in the genes for either the a- or the b-subunit of the cone photoreceptor cGMP gated channel result in complete colorblindness with indistinguishable phenotypes.

Molecular Pathogenesis of Fibrillin-1 Mutations Involving the Carboxy Terminal Domain. *D.D. Wallis, A. Cantu, E.A. Putnam, J. Grossfield, S. Cao, D.M. Milewicz.* Int Med/Med Gen, Univ Texas Medical Sch, Houston, TX.

Marfan syndrome is an autosomal dominant condition that results from mutations in the FBN1, which encodes fibrillin-1 (fib-1), an extracellular matrix (ECM) protein. Fib-1 is synthesized as a proprotein that is proteolytically processed in the C-terminal domain, and then deposited into the ECM. Surprisingly, FBN1 nonsense mutations have been identified distal to the C-terminal cleavage site. We have also identified 2 unrelated Marfan patients (P007 and P029) with genomic mutations causing exon 64 splicing error, which lead to truncation of profib such that the entire C-terminal domain is deleted. We investigated how these FBN1 mutations disrupt fib-1 incorporation into the ECM. Dermal fibroblasts were explanted from P007 and P029, and from a patient with a previously reported nonsense mutation (8236delAG) distal to the C-terminal processing site. Pulse-chase analysis of profib-1 demonstrated that the truncated profib-1 was inefficiently secreted in all 3 cell strains. Delay in the migration in the SDS-PAGE gels of the truncated profib-1 implied that the retained profib-1 was over-glycosylated. Fib-1 deposition into the ECM was also diminished to less than 5% that of control cells. To determine if the overglycosylation disrupted the deposition of fib-1 in the matrix P007 and P029 were metabolically labeled in the presence of increasing amounts of tunicamycin, which blocks N-linked glycosylation. These studies indicated that the amount of fib-1 deposition into the ECM increased in both the patient cells and control cells at low doses of tunicamycin. These results indicate that not only the C-terminal domain of profib-1 is important for efficient secretion but also that fib-1 incorporation into the ECM is sensitive to the amount of N-linked glycosylation.

An autosomal dominant disorder similar to Huntington's disease is associated with a CAG trinucleotide repeat expansion. *R.L. Margolis¹, E. O'Hearn^{2, 4}, A. Rosenblatt¹, J. Troncoso³, S.E. Holmes¹, M.L. Franz¹, M. Sherr¹, C. Callahan¹, J. Hwang¹, C.A. Ross^{1, 4, 5}.* 1) Dept. of Psychiatry; 2) Dept. of Neurology; 3) Dept. of Pathology; 4) Dept. of Neuroscience; 5) Program of Cellular and Molecular Medicine, Johns Hopkins Univ Sch Med, Baltimore, MD.

We have ascertained a large pedigree with a disorder resembling Huntington disease (HD). Inheritance is autosomal dominant, with minimal evidence of anticipation, onset in the 4th-5th decade, and progression over about 10 years to a bedridden state. The phenotype is characterized by weight loss, ataxia, chorea, bradykinesia, dysarthria, dementia, hyperreflexia, tremor, and psychiatric symptoms. Neuropathological examination of one brain revealed prominent striatal atrophy, with a loss of medium sized neurons in a dorsal to ventral gradient. A CAG trinucleotide repeat expansion, detected by the repeat expansion detection (RED) method, segregates with affected status and is not accounted for by any of the known CAG repeat expansions. Determining the genetic etiology and molecular pathogenesis of this familial disorder may provide important insights into the pathophysiology of HD and other neurodegenerative disorders.

Mal de Meleda: Candidate gene analysis on chromosome 8q24.3 and identification of genetic and clinical heterogeneity. *H.C. Hennies¹, G.G. Lestringant², H.P. Stevens³, B. Hinz¹, P.M. Frossard², I.M. Leigh³, A. Reis¹.* 1) Dept of Molecular Genetics, Max-Delbrück Center, Berlin, Germany; 2) Tawam Hospital and Faculty of Medicine and Health Sciences, Al Ain, United Arab Emirates; 3) Center for Cutaneous Research, St Bartholomew's, London, UK.

Mal de Meleda (MDM) is a congenital, autosomal recessively inherited disorder of cornification characterized by palmoplantar hyperkeratosis transgressing to the dorsal parts of hands and feet. Hyperhidrosis, perioral erythema, lichenoid plaques, and nail abnormalities were described as further symptoms.

We have analyzed three families with MDM, one large Palestinian family and two Arab families, all of these consanguineous, for linkage. In these families, the gene was localized on chromosome 8q24.3. This localization is consistent with a recent report, which described mapping of the gene in two families with MDM from Algeria telomeric to D8S1727. Here we present the refined localization of the gene to an interval of less than 2 cM between D8S1751 and AFMa128xh5. Within this region, the gene encoding E48 antigen has been mapped. This is a small GPI-anchored membrane protein homologous to a 20-kD bovine desmosomal protein and exclusively expressed in transitional keratinocytes. It has been shown that the protein is involved in cell-cell adhesion. Therefore, the gene is a promising positional candidate for the MDM gene. Analysis by direct sequencing of coding parts of the gene and its promoter, however, revealed no change in the gene so far. Further investigations of transcripts from keratinocytes of MDM patients and immunohistochemical analyses of the gene product are currently in progress.

Linkage analysis in three further Arab families with a very similar phenotype, however, excluded 8q24 in these patients. We conclude that either MDM is genetically heterogeneous, or there is at least one other phenotype resembling MDM but caused by mutations in a different gene. Additional families with MDM from various origins are investigated to further cut down the region, or to identify additional families with the MDM variant, which could help to localize the gene for this as yet unknown phenotype.

Recessive Multiple Epiphyseal Dysplasia (rMED): phenotype delineation in twelve individuals homozygous for *DTDST* mutation R279W. A. Superti-Furga¹, J. Hecht², S. Unger², W. Cole², B. Hamel², G. Bellus², M. Classen², M. LeMerrer², B. Zabel², L. Langer², J. Spranger², D. Cohn², D. Sobetzko¹. 1) Metab Mol Dis, Univ Child Hosp, Zurich, Switzerland; 2) Academic Institutions in Houston, Los Angeles, Toronto, Nijmegen, Denver, Bremen, Paris, Mainz.

Background, Aim: R279W is the most common mutation in *DTDST*, the gene associated with achondrogenesis 1B / atelosteogenesis 2 / diastrophic dysplasia. Following observation of R279W homozygosity in a man with MED and normal stature (J Med Genet 36:621-624, 1999), we have found R279W homozygosity in 12 further MED individuals from 7 families. This study was aimed at defining the phenotype of rMED in this homogeneous sample population.

Methods: Patients ascertained because of clinical features similar to the published case or because of a diagnosis of MED with no mutation found in *COMP* and collagen 9 genes. *DTDST* mutation analysis by PCR and direct sequencing. Charts and X-rays analysed retrospectively using an *ad-hoc* questionnaire. **Results:** 4 male, 8 female pts.; age at evaluation, 9 to 46 yrs; all Caucasian origin; no parental consanguinity. Abnormal findings at birth in 4/12 cases (clubfoot 3/12; cleft palate 1/12; no ear cysts or hitch-hiker thumbs). Diagnosis of skeletal dysplasia made at birth in 2/12 cases, in childhood or adolescence in 10/12 cases. Leading symptoms/signs: joint pain (8/12 cases), hand/foot deformities (4/12), scoliosis/contractures (1/12 each). Chronic joint pain in 10/12 cases (hips, knees, wrists, fingers). Stature below -2SD for age in 0/2 prepubertal and in 4/10 postpubertal patients. Radiographic pattern of "flat epiphyses" MED (Ribbing) with mild brachydactyly and early coxarthrosis. Double-layered patella in 4/5 patients. **Conclusions:** Homozygosity for R279W in *DTDST* produces a distinct MED phenotype. Clinical signs may be present at birth but the diagnosis of skeletal dysplasia is made usually in childhood or adolescence following presentation with joint pain and/or hand/foot deformity. Stature is normal or mildly shortened. Arthritis and joint pain are common. *DTDST*-associated rMED should be distinguished from dominant MED forms because of different counselling implications.

The effect of telethonin deficiency in LGMD-2G and its expression in other forms of muscular dystrophies and congenital myopathies. *M. Vainzof^{1,2}, E.S. Moreira¹, M.R. Passos-Bueno¹, G. Faulkner³, G. Valle³, E. Zanoteli⁴, J. Gurgel-gianneti², A.F. Ribeiro¹, H.C.A. Silva⁵, A.M.C. Tsanaclis⁵, M. Zatz¹.* 1) Center for the Study of the Human Genome, Dept. Biology, IBUSP, University of Sao Paulo, Sao Paulo SP, Brazil; 2) Dept. of Neurology, FMUSP; 3) International Center for Genetic Engineering and Biotechnology, Trieste, Italy; 4) Dept. of Neurology, UNIFESP; 5) Dept. of Patology, FMUSP, Sao Paulo, Brazil.

Telethonin is a sarcomeric protein of 19 kD, possibly localized to the Z-disc of adult striated skeletal and cardiac muscles. Recently, we have shown that mutations in the telethonin gene cause LGMD2G, a mild form of autosomal recessive LGMD, mapped to 17q11-12 in three Brazilian families. This was confirmed through Immunohistochemistry and western blot analysis using an anti-telethonin antibody in muscle of affected patients, that showed no protein expression (Moreira et al., Nature Genetics 24:163-166, 2000). Here, we studied the sarcomeric integrity of muscle fibers in patients with LGMD2G, through double analysis for telethonin and α -actinin-2 and electron microscopy (EM) analysis, as well as the possible interaction of telethonin with the other proteins responsible for several forms of AR-LGMD (2A, 2B, SGpathies), DMD, and nemaline myopathy (NM). Muscle from patients with LGMD2G showed normal expression of dystrophin, sarcoglycans, dysferlin and calpain proteins. Immunofluorescence analysis for α -actinin-2 showed a cross striation pattern, suggesting that at least part of the Z-line of the sarcomere is preserved. Ultrastructural analysis confirmed the maintenance of the integrity of the sarcomeric architecture. Telethonin was clearly present in the rods, in muscle fibers from patients with nemaline myopathy, confirming its localization in the Z-line of the sarcomere. The analysis of telethonin on muscle biopsies from patients with LGMD2A, LGMD2B, SGpathies and DMD showed normal localization, suggesting that the deficiencies of calpain, dysferlin, sarcoglycans and dystrophin do not seem to alter telethonin expression. Financial Support: FAPESP, PRONEX, FFM, ABDIM.

Mutation spectrum within the human JAG1 gene in Alagille syndrome. *A. Roepke, A. Kujat, M. Graeber, I. Hansmann, J. Giannakudis.* Institut für Humangenetik und Medizinische Biologie, Martin-Luther Univ, Sachsen-Anhalt, Germany.

Alagille syndrome (AGS, MIM 118450, McKusick, 1998) is an autosomal dominant disorder characterized by liver disease with heart, skeletal, ocular and facial abnormalities. The gene JAG1 on chromosome 20p12 has been identified as responsible for AGS. JAG1 encodes a ligand in the Notch signaling pathway, that plays an important role in cell fate determination. JAG1 spans 36 kb, comprises 26 exons and encodes a 1218 amino acid protein with several domains and high interspecies conservation. In our survey of 91 AGS patients we evaluated frequency and type of mutation in the JAG1 gene by SSCP and sequencing as well as microsatellite deletion analysis. Deletions involving larger portions of the gene were observed in 7 patients (7.7%). Of the remaining 84 patients, 60 (71%) have mutations within JAG1 including 15 (18%) small deletions, 6 (7%) small insertions, 13 (15%) nonsense, 14 (17%) missense and 12 (14%) splice site mutations. Most of the mutations (88%) are expected to result in a truncated protein thus leading to loss of functional domains that are responsible for the interaction with the Notch receptor. This observation is consistent with haploinsufficiency of JAG1 as well as with a dominant negative effect. One mutation was found in exon 26 within the transmembrane domain. This mutation should reduce the hydrophobicity of this region. 8 of 14 missense mutations occur in exons 4-6 and show an exchange of amino acids which are highly conserved from *Drosophila* to human. Yet the mutations are distributed over the entire gene evidence is provided for a mutational bias in exon 5 and 17. To date no mutation was found in exon 8. In 25 out of 41 cases (61%) a de novo mutation was found. In 16 families (39%) the mutation could be determined as maternal (n=10) or paternal (n=6). According to our analysis up to 9% of the cases are due to genetic mosaicism. Because mosaicism was also detected in clinically mildly affected patients with AGS, this has to be taken into account in genetic counselling. Supported by the DFG.

Clinical and pathological variability of congenital myopathies caused by mutations in skeletal muscle alpha-actin.

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Skeletal muscle a-actin gene (*ACTA1*) mutations have recently been described in patients with nemaline rod myopathy and actin myopathy. These and other congenital myopathies are characterized by muscle weakness of variable severity and abnormal structural findings in muscle fibers. All the *ACTA1* mutations initially described were missense changes, and most were sporadic with a clinically severe phenotype. One case each of dominant and recessive transmission were also observed. Here we describe six novel *ACTA1* missense mutations identified in a screen of twenty patients with nemaline rod myopathy. In addition, we found a single base pair deletion leading to a truncated protein product in a patient with severe, undefined congenital myopathy. Five of six missense mutations occurred in exon 3 with one in exon 6. In the nemaline myopathy patients, *de novo* sporadic dominant changes were found in two patients with moderate to severe phenotypes. Four mutations were identified in families with dominant inheritance of milder phenotypes including at least one instance of nonpenetrance. In the undefined congenital myopathy patient with a frameshift, the muscle biopsy showed marked variation in fiber size with atrophic fibers containing cytoplasmic bodies on trichrome stain. Electron microscopy revealed abnormal basement membrane structures and focally condensed Z disc materials. This infant had severe hypotonia and respiratory insufficiency and died in the second month of life. These data indicate that *ACTA1* mutations cause a wider range of clinical and pathological phenotypes than previously suspected. The finding of *ACTA1* mutations in roughly one third of patients screened suggests that skeletal muscle actin abnormalities are a common cause of nemaline myopathy. Finally, the identification of a frameshift mutation in a patient without nemaline rods broadens the range of congenital myopathies that may result from *ACTA1* mutations.

Critical role of the interface between cell membrane and extracellular matrix is demonstrated by multi-organ abnormalities in Laminin-2 deficient mouse models for congenital muscular dystrophy. *D.M. Pillers^{1,2}, G.K.*

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Introduction: Muscular dystrophies are, by definition, best known for their muscle phenotypes. Lesser known are the non-muscle manifestations that the disorders also include. **Purpose:** We sought to characterize the distribution of non-muscle phenotypes in mouse models of muscular dystrophy as a marker for a functional role of proteins implicated in muscular dystrophy in a variety of organ tissues. **Methods:** We studied the *dy* mouse, a severely affected model for merosin-deficient congenital muscular dystrophy. This mouse has absence of expression of the protein subunit laminin- α 2, which results in aberrant expression of the trimer Laminin-2 and disrupts the interaction between cell and extracellular matrix (ECM) in myocytes. We hypothesized that clinical evidence of organ pathology would suggest a role for Laminin-2 and its interaction with the ECM in other cell lineages. Several physiologic tests were employed, analogous to clinical screening tests used in man. **Results:** A hypertrophic cardiomyopathy which progresses to a dilated cardiomyopathy was detected. Mild to moderate sensorineural hearing loss was found. A defect in retinal electrophysiology was identified. In heterozygous animals, an obese body habitus was found. Obesity, which is common in muscular dystrophy patients, suggests involvement of the neurohypophysis and the possibility that it may be a primary process rather than a secondary effect of the disease. **Conclusions:** Proteins defective in muscular dystrophies enjoy a wide tissue distribution which may reflect a key-role in multiple organ functions. The protein complex spanning from the actin cytoskeleton to the extracellular matrix, via dystrophin, the dystrophin-associated proteins, and Laminin-2, is well-defined in myocytes but further studies are indicated to delineate its composition and contribution in other cell lineages.

Clinical and Genetic Diversity in Autoimmune Lymphoproliferative Syndrome (ALPS), an Inherited Disorder of Lymphocyte Apoptosis. *C.E. Jackson¹, L.L. Bi², R.E. Fischer¹, J. Davis¹, J. Niemela³, J.K. Dale², T.A. Fleisher³, M.J. Lenardo², S.E. Straus², J.M. Puck¹.* 1) GMBB/NHGRI; 2) Lab of Clin Invest and Lab of Immunol/NIAID; 3) Clinical Center Clinical Lab; NIH, Bethesda, MD.

Autoimmune Lymphoproliferative Syndrome (ALPS) is a disorder of immune homeostasis and tolerance. Defective lymphocyte apoptosis in ALPS leads to accumulation of lymphocytes, including ab CD4-/CD8- T cells, and autoimmunity. Most patients with ALPS have mutations in TNFRSF6, which encodes Fas, the receptor for the major apoptosis pathway in mature lymphocytes. Mutations in other apoptosis genes can also cause ALPS, therefore, ALPS is subdivided into: type Ia, mutant Fas, type Ib, mutant Fas ligand, type II, mutant caspase 10, and type III, ALPS with currently undetermined genotype. Of 43 confirmed ALPS probands in the NIH cohort, 31 (72%) had type Ia, 2 (5%) type II, and 5 (12%) type III. Penetrance of ALPS Ia in NIH kindreds, in contrast to many autoimmune diseases, was greater for males (41/57; 72%) than for females (26/51; 51%) ($p < 0.05$). Age at presentation was similar in males and females. 22 of the 31 (67%) TNFRSF6 mutations in ALPS type Ia patients affected the intracellular death domain of Fas, produced the strongest dominant inhibition of apoptosis and were associated with greater penetrance of ALPS features in mutation-bearing relatives (86%) as compared to extracellular mutations (46%) ($p < 0.001$). Significant morbidity associated with ALPS type Ia (splenectomy, autoimmunity requiring treatment, and/or lymphoma) was also increased in mutation-bearing relatives with intracellular mutations (45%) compared to mutation-bearing relatives with extracellular mutations (8%) ($p < 0.001$). Our studies demonstrate the clinical diversity and genetic heterogeneity of ALPS and suggest that apoptosis defects may contribute to autoimmunity and lymphoma.

Russell-Silver Syndrome: Establishment of a possible critical region on 7p14 and characterization of putative candidate genes. *K. Nakabayashi¹, B.A. Fernandez¹, R. Edge¹, I. Teshima², C. Shuman¹, R. Weksberg¹, K. Mitsuya³, M. Meguro³, M. Oshimura³, S.W. Scherer¹.* 1) Dept. of Genetics; 2) Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada; 3) Dept. of Molecular and Cell Genetics, Faculty of Medicine, Tottori University, Tottori, Japan.

Russell-Silver syndrome (RSS) is a form of congenital dwarfism characterized by severe growth retardation and variable dysmorphic features. RSS is considered to be an imprinting disease, because 10% of sporadic patients demonstrate maternal uniparental disomy (mUPD) of chromosome 7. Although 3 genes on chromosome 7 (*MEST*, *g2COP* and *GRB10*) are shown to be imprinted in human or mouse, several lines of evidence exclude their involvement in RSS, suggesting the existence of additional imprinted gene(s) on chromosome 7 which contribute to the disease. Two RSS patients were identified with de novo cytogenetic abnormalities involving the short arm of chromosome 7. One had a partial duplication [46, XX, dup(7)(p12p14)] and the second contained a paracentric inversion [46, XY, inv(7)(p14p21)]. Based on regions of overlap of the rearrangement breakpoints determined by fluorescence in situ hybridization (FISH) mapping, a putative critical interval approximately 1Mb in size has been established. A BAC/PAC contig covering the interval was constructed. The genomic DNA sequences from the interval were subjected to database comparisons and gene prediction programs. Seven candidate genes have been identified so far. Allelic expression of these genes were examined by RT-PCR using mouse A9 somatic cell hybrids containing paternal or maternal human chromosome 7, in which the imprinting of *MEST* was maintained. All seven genes showed biallelic expression in A9 hybrids, although preliminary data indicated one of them, *CDC2L*, might show paternal expression bias. Moreover, this gene was nearest to the breakpoints. The exon-intron structure of *CDC2L* and the other positional candidate genes were determined for mutation screening in RSS patients.

Unequal crossovers in a 2 kb recombinational hotspot as a cause of NF1 microdeletions*NF1. E.H. Legius¹, C. Lopez-Correa¹, H. Brems¹, T. Deraedt¹, C. Lazaro², M. Clementi³, D. Dooijes⁴, U. Moog⁵, P. Marynen¹, J.P. Fryns¹.*
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We performed molecular studies in 38 NF1 microdeletion patients. Twenty-two out of 27 microdeletions where the parental origin could be studied were of maternal origin (81%) and 5 were of paternal origin. Thirty-seven of the 38 microdeletions have a 1.5 Mb size with breakpoints in flanking paralogous sequences (NF1-REPs). These paralogous sequences are >100 kb long and represent duplicated sequences in a direct orientation with 98% homology. At least 5 expressed sequences are present in the flanking paralogous sequences. Additional paralogous sequences are present inside the NF1 microdeletion, more telomeric on 17q24 and on 19p. In 7 informative 3-generation families with NF1 microdeletions of maternal origin an interchromosomal meiotic recombination could be demonstrated as well as in two informative cases of paternal origin. Further molecular analysis of the 37 cases with breakpoints in the flanking paralogous sequences showed that 19 microdeletion breakpoints clustered in a 2 kb region. There was no specific structure present at the breakpoints responsible for the meiotic recombination process. Frequent conversions were observed in the breakpoint region as was previously described in CMT1A and HNPP. These data show that about 50% of NF1 microdeletions originated from an unequal recombination between paralogous sequences on the homologous chromosomes 17 during the first meiotic division. The unequal meiotic crossovers cluster in a 2 kb region and occur more frequently during female meiosis than during male meiosis. This recombinational process interrupts 1 of the 5 expressed sequences in the flanking NF1-REPs and deletes 4 expressed sequences located in the telomeric NF1-REP.

Investigation of Three-Nucleotide-Repeat expansion in Iranian SBMA patients. *M. Houshmand¹, A. Ghashghaei¹, Z. Pourmahdi¹, M. Siavash², A. Zamani².* 1) Medical genetics unit, National Research Center for Genetic Engineering and Biotechnology, Tehran, Iran; 2) Rassol Akram Hospital, Tehran, Iran.

X-Linked recessive Spinal and bulbar muscle atrophy is an adult onset motor neuron disease which occurs due to an expansion of three-nucleotide-repeat especially CAG in the first exon of Androgen receptor gene. The number of CAG repeats is 19-25 in normal healthy individuals and 40-52 in affected individuals. The size of Androgen receptor gene is one of the severity and progression rate of SBMA phenotypes. Our proband was a 58-years-old man with gynecomastia, progressive proximal muscle atrophy, regurgitation, fasciculation and decreasing libido and potency. He showed the clinical signs at the age of 35. He belongs to the third generation of this pedigree. Large pedigree was investigated by clinical examination. In this pedigree eight persons were affected. One affected person married with his cousin and their daughter is suspected to SBMA. In this study we examined the expansion of CAG repeats in blood samples of four generations. PCR products were run on 2% agarose gel. We have identified a large fragment in the affected males by PCR in this family comparison with control healthy individuals. Two carrier females were identified in this pedigree. The PCR product of carrier showed two bands. Shorter band the same as healthy one and larger band as affected one. The resulting fragments were separated by electrophoresis in polyacrylamide gels. We investigate the expansion of three repeat in four generations.

Characterization of a Novel Member (SLC19A3) of the Reduced Folate Carrier (SLC19A1) and Thiamine Transporter (SLC19A2) Gene Family. *J.D. Eudy, O. Spiegelstein, R.C. Barber, R.H. Finnell.* Center for Human Molecular Gen, University of NE Medical Cent, Omaha, NE.

Statement of Purpose: The purpose of this study was to identify and characterize novel members of the SLC19A gene family of micronutrient transporters. Methods: Expressed sequence tag (EST) databases were screened using the BLAST program and the protein sequence of the SLC19A1 gene. Partial gene sequences corresponding to novel SLC19A family members were identified using this bioinformatic strategy. PCR primer pairs were used to screen human and mouse cDNA libraries. Northern Blot analysis as well as semiquantitative RT-PCR was performed. The human and mouse genes were mapped by radiation hybrid mapping. Summary of Results: We present the identification, isolation, and characterization of the human and mouse genes encoding the third member (SLC19A3) of the reduced folate carrier-1 family of micronutrient transporters. SLC19A3 contains 12 putative transmembrane domains and likely transports a micronutrient of the B-vitamin family. Human SLC19A3 exhibits widespread expression with the highest levels present in placenta, kidney, and liver. Mouse SLC19A3 is expressed at high levels in kidney, brain, lung with minor expression in other tissues. The human and mouse orthologue map to their respective chromosomal locations on human chromosome 2q37 and the corresponding syntenic region of central murine chromosome 1. Although the specific B-vitamin transported by SLC19A3 is as yet unknown, insight into the transport specificity of SLC19A3 may be provided by the chromosomal location of the mouse SLC19A3 gene. Mouse SLC19A3 maps to central chromosome 1 in a region previously defined as a seizure susceptibility locus in the DBA/2J mouse strain. Independent evidence has suggested that the seizure susceptibility of DBA/2J is associated with abnormalities of vitamin B-6 (pyridoxamine) metabolism. To date, a pyridoxamine transporter has not been identified in higher eukaryotes. These studies identify a novel human micronutrient transporter gene (SLC19A3) and establish its murine orthologue as a candidate gene for the seizures observed in the DBA/2J mouse strain.

Factor V Leiden mutation and factor II Leiden mutation in a healthy subject without thrombosis antecedent. *S. Guermazi, A. Bahlous, N. Labbane, S. Abdelhak, K. Dellagi.* Pasteur Institut of Tunis, Tunis-Blvdr, Tunis, Tunisia. Laboratoires d'Hématologie et d'Immunologie.

Two new markers of inherited thrombophilia have been described last years; factor V Leiden mutation (FV Leiden) (R506Q) which is responsible for about 95% of activated protein C resistance (APCR), and factor II Leiden (FII Leiden) which corresponds to G20210A mutation in a non coding region of prothrombin gene. The frequencies in normal populations of these two markers of thrombophilia are respectively 0-15% and 1-4% for FV Leiden and FII Leiden. In groups of patients with past history of thrombosis the reported frequencies are respectively 20-50% and 6-9% for FV Leiden and FII Leiden. We have studied 80 normal Tunisian subjects for APCR by a coagulometric method (modified test of Dahlback) and we found 10% APCR subjects. Direct sequencing confirmed the FV Leiden mutation in all APCR subjects. Factor II Leiden mutation was studied as follows; amplification by PCR of exon 14 and the 3'UTR adjacent region, enzymatic digestion with MspI which creates a restriction site specific for G20210A mutation, and MspI as internal control of digestion. A preliminary screening of 44 subjects of this group for FII Leiden showed a positive subject, confirmed by direct sequencing. This normal subject was also heterozygous for FV Leiden mutation. No thrombosis antecedents were found in this 44-year-old subject. The presence of two genetic markers of thrombophilia in a healthy normal control shows that these abnormalities, even when associated, must be considered by clinicians only as risk factors of thrombosis and not as morbid mutations.

Molecular characterization of human cytochrome P4501B1 (CYP1B1) gene in Italian PCG patients. G.

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Primary congenital glaucoma (PCG) is an autosomal recessive disorder associated with undefined developmental defects in the anterior eye chamber angle and trabecular meshwork. PCG is usually diagnosed at birth. Two chromosomal locations GLC3A (2p21) and GLC3B (1p36) were reported to be linked with PCG. The human CYP1B1 gene, coding for cytochrome P4501B1 and expressed in trabecular meshwork, has been identified within the GLC3A locus. Several mutations have been described for CYP1B1 gene in segregating PCG families of various origins. In Saudi Arabian populations three common mutations account for 91% of all PCG chromosomes revealing the presence of a founder effect. In our study, we collected 28 families from distinct geographical areas of Italy. We constructed haplotypes with polymorphic markers (D2S1325, D2S2186, D2S1346, D2S1356) flanking CYP1B1 gene. Mutation analysis was carried out by direct sequencing of all the CYP1B1 coding exons. Haplotypes analysis showed no evidence of Identity by Descent sharing. Four mutations were identified. Three of them were already described: 1410del13 and P437L, present only in Turkish populations, and G61E, found in Turkish and Saudi Arabian populations. The novel mutation here identified, resulting in the substitution of Alanine with Aspartic acid (A106D), was detected in compound heterozygous with 1410del13 in a patient of Northern Italy. The 1410del13 was the most frequently observed mutation in our chromosomes (7%), present in different Italian regions. The geographical distribution of these molecular defects in Italian population is thus not consistent with the presence of any founder effect. In addition, the low incidence (14% of analyzed chromosomes) of a molecular defect in CYP1B1 suggests genetic heterogeneity in the PCG pathogenesis within Italian populations. A larger sampling is being achieved to confirm our findings. These results are instrumental to implement an early diagnosis program.

A novel TBX3 frameshift mutation in a patient with Ulnar-Mammary syndrome. *A. Jobard, M. Le Merrer, S. Audollent, J. Augé, J. Martinovic, A. Munnich, M. Vekemans, T. Attié-Bitach.* Dep de genetique INSERM-U393, Hopital Necker-Enfants Malades, Paris, France.

Ulnar-Mammary syndrome is characterized by ulnar ray anomalies and hypoplasia or aplasia of the mammary glands and nipples. Other clinical features include anomalies of axillary apocrine glands, urogenital system, anal anteposition or imperforation, lacrymal duct atresia, nail dysplasia, hypodontia and cleft palate. Ulnar- Mammary syndrome has been ascribed to TBX3 gene mutations and 10 different mutations have been reported so far (Bashmad,1997 and 1999). Here, we describe a novel TBX3 mutation in a patient with clinical feature of Ulnar-Mammary syndrome. A girl presented with severe hand anomalies and aplasia of the right mammary gland and nipple. She had right hand oligodactyly with complete absence of the 3rd, 4th and 5th digit and ulna, and a dimple of the elbow. On the left side, she had ulnar ray aplasia with severe hypoplasia of the 5th digit. She also had aortic stenosis. We sequenced the complete coding sequence of the TBX3 gene in this patient and identified a one bp deletion in exon 1, at position 135 of the cDNA sequence. This frameshift mutation is predicted to cause a premature termination of the protein 65 codons downstream from the deletion, and therefore a truncated protein lacking the entire T-box. This study further confirms the involvement of TBX3 in Ulnar-Mammary syndrome. Interestingly, another gene belonging to the T-box genes family, TBX5 is responsible for a radial ray and heart defect syndrome, Holt-Oram syndrome, suggesting that these two genes may play a role in the specification of the dorsal/ventral axis of the forelimb. Ulnar ray defects are rare in human, and other patients with syndromic ulnar ray defects and/or hypoplasia of the mammary gland are being screened for TBX3 gene mutations.

Progress towards the identification of the IRID1b locus at chromosome 6p25. *F. Mirzayans¹, D.B. Gould¹, M. Thorne², V. Raymond³, T. Jordan⁴, S. Bhattacharya⁴, M.A. Walter¹.* 1) Ophthalmology/Medical Genetics, Univ Alberta, Edmonton, AB, Canada; 2) Genome Sequence Centre, BC Cancer Research Centre, Vancouver, BC, Canada; 3) CHUL Research Centre, Univ Laval, PQ, Canada; 4) Institute of Ophthalmology, London, England.

IRID1, a major locus for anterior segment development, has been previously mapped to chromosome 6p25. Mutations in FOXC1, a member of the Forkhead/Winged-Helix gene family of transcription factors also mapping to 6p25, have been observed in families and sporadic patients affected with the related ocular disease Axenfeld-Rieger malformation. However, analysis of additional polymorphic markers and intragenic recombination events in FOXC1 observed in two of our large families affected with anterior segment dysgenesis linked to 6p25 point to the existence of another locus, IRID1b, distal to the FOXC1 gene at 6p25. Analyses of six additional newly derived polymorphic markers have restricted the IRID1b critical region to an interval less than 500 kb in size at distal 6p25. A second forkhead gene, FOXF2, located approximately 100 kb telomeric to FOXC1 has been excluded as an IRID1b candidate gene since direct sequencing efforts have not detected any disease causing alterations in FOXF2 in our patients. Characterization of the expression profiles and mutational analyses of ESTs and predicted genes from in silico analyses of genomic sequence data of the IRID1b locus are underway. Identification of the underlying molecular defect in IRID1b patients will not only aid in the management and treatment of the glaucoma that is the most serious consequence of IRID1b, but give fundamental insight into how the neural crest is regulated and functions in embryogenesis.

Apert syndrome with a second novel mutation in the *FGFR2* gene. *M.M. Nezarati*¹, *L. Steele*², *S.J. Withers*¹, *P.N. Ray*^{2,3}. 1) Div Clinical/Metabolic Genetics; 2) Dept Paediatric Lab Medicine; 3) Genetics & Genomic Biology, The Hospital for Sick Children, Toronto, ON, Canada.

Apert syndrome is a genetic disorder whose main features include severe craniosynostosis, ocular proptosis and hand & foot syndactyly. Most cases are sporadic and are due to recurrent mutations found in the Fibroblast Growth Factor Receptor 2 (*FGFR2*) gene. We report a case of a baby girl born at term with clinical features most resembling a mild Apert or Pfeiffer syndrome. The proband was born at term by Cesarean section for fetal decelerations. She was noted to have a turricephalic skull, with mild proptosis and mid-face hypoplasia. She had syndactyly of digits 2, 3 and 4 bilaterally on both of her hands and feet. Her thumbs and great toes were broad. The remainder of the exam was essentially normal. Molecular genetic testing using direct sequencing revealed the presence of the recurrent P253R mutation in the *FGFR2* gene. In addition, a C®T substitution was detected 30bp downstream, which would predict a missense substitution of a proline for a leucine at amino acid 263. Both of these amino acids are highly conserved among all four *FGFR* genes and are located within the linker region of *FGFR2* connecting the IgII and IgIII domains. This linker region is responsible for ligand binding. Previously reported protein function studies have shown that the P253R mutation confers a 2-fold increase in FGF2 binding affinity compared with wild-type *FGFR2*. Since the significance of this second sequence alteration on protein function is not known, parental bloods were obtained to determine which parent carries the P263L substitution. However, neither parent was found to carry the change and therefore, both mutations appear to be de novo (>99.9% probability of paternity). Molecular cloning studies are underway to determine if the P253R and P263L mutations are in cis or in trans in the *FGFR2* gene. We suspect that the P263L substitution may have had an impact on ameliorating the phenotypic manifestation of the recurrent Apert mutation in this child. Further studies are required in order to determine the functionality of the altered protein.

Towards the gene defect causing Meckel syndrome. *M. Kyttala*^{1,2}, *P. Paavola*², *R. Salonen*³, *L. Peltonen*^{1,2}. 1) Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA; 2) Department of Human Molecular Genetics, National Public Health Institute, Helsinki, Finland; 3) Department of Obstetrics and Gynecology, Helsinki University Hospital.

Meckel Syndrome (MKS; MIM 249000) is an autosomal recessive congenital malformation syndrome leading to death soon after birth. The clinical phenotype of MKS includes central nervous system malformations, occipital meningo-encephalocele, polycystic kidneys, cystic and fibrotic changes in the liver, and polydactyly. In Finland the prevalence of MKS at birth is estimated to be 1:9000. MKS has been mapped in Finnish population into chromosome 17q22-23, but the locus heterogeneity in study samples from other populations has also been reported with a linkage to chromosome 11q13 (Roume et al., 1998). We have restricted the critical MKS region to 700kb on chr 17q in Finnish families using linkage disequilibrium and identification of one ancient haplotype in Finnish disease alleles. We have constructed a multiple coverage physical contig of PAC and BAC clones over this region and 75% of the sequence of this critical region is now available in gene bank databases. Two repeat markers, separated by 70 kb on the critical region show almost complete allelic association suggesting the location of MKS gene in the immediate vicinity of them. We have sequenced all known genes on the critical region in MKS patients, but so far have not been able to show any nucleotide alteration in the obtained sequences in MKS patient. Currently we are analyzing two novel cDNAs with homologies to homeobox genes and transcription factors. These and other novel genes on this region should eventually expose the MKS gene.

Analysis of gene expression in a mouse model for Norrie disease by cDNA subtraction and microarrays. *S. Lenzner, S. Prietz, S. Feil, R. Schulz, H.H. Ropers, W. Berger.* Max-Planck-Institute for Molecular Genetics, Berlin, Germany.

Norrie disease (ND) is an X-linked recessive trait characterized by congenital blindness, mental retardation and deafness. Mutations in the ND gene, which codes for a growth factor-like protein of unknown function, were also found in patients with exudative vitreoretinopathy, retinopathy of prematurity, and Coats disease, three distinct clinical entities which are not associated with mental or auditory defects. To unravel the function of this gene and the disease processes on the molecular level, cDNA subtraction and cDNA microarrays were employed to monitor gene expression profiles in the eyes of ND knockout mice during disease progression. Subtraction libraries were generated from RNA templates of age-matched wildtype and knockout mice at 4 weeks and 2 years in order to identify early (primary) and late (secondary) alterations in the gene expression patterns, respectively. More than 3000 clones from the subtraction library at 2 years were spotted onto glass slides and screened with RNA-derived targets from wildtype versus knockout mice. About 1300 clones gave differential signals which are being verified by Northern blot analysis. Subsequently, differential clones are sequenced. In this way, we have identified numerous eye/retina-specific transcripts, which are almost absent in the RNA of knockout mice at 2 years of age. Some of these are photoreceptor cell-specific, including the phosphodiesterase beta and gamma subunits, blue cone pigment, phosducin, arrestin, recoverin, and the alpha subunit of the cGMP-gated cation channel. In younger knockout mice, however, all of these genes were found to be expressed, indicating that photoreceptor cells are affected relatively late in ND mice. In contrast, the RS1 gene which is mutated in patients with X-linked juvenile retinoschisis is already down-regulated in early stages of the disease. Detailed characterization of the subtraction library from 4 weeks old mice will reveal which genes are primarily involved in the disease process.

Molecular Analysis of Lamellar Ichthyosis in nine Tunisian families. *N. Belghith¹, M. Chaabouni^{1,2}, F. Zeglaoui³, N. Smaoui^{1,2}, R. Mrad^{1,2}, MR. Kammoun³, H. Chaabouni^{1,2}.* 1) Laboratory of Human Genetics. , Medical school Tunis,Tunisi, Tunis, Tunis, Tunisia; 2) Department of Hereditary Diseases .Charles Nicolle hospital. Tunis,Tunisia; 3) Department of Dermatology Charles Nicolle hospital. Tunis,Tunisia.

Lamellar ichthyosis (LI) is an inherited autosomal recessive disorder of cornification. The disease was shown to be genetically heterogeneous since three different genes have been described : TGM 1 gene on14q11.2, a second locus on 2q33- 35 in Moroccan families and recently a locus on 19p 12-q12 region. We tested 9 Tunisian families with autosomal recessive LI including 20 affected members for 4 chromosome 2 markers (D2S325, D2S157, D2S143, D2S137)and 3 chromosome 14 markers (D14S972, D14S64, D14S264). Segregation analysis of 100 chromosomes didnt conclude for linkage with chromosome 2. Lod scores analysis allowed us to exclude this locus. Analysis of chromosome 14 markers gives positive lod score (=3.62) for 6 families, and negative lodscore for remaining families. More individuals are required for linkage conclusion. Tunisian LI families seem genetically different from Moroccan ones .Regarding our results and because of high number of loci, we have to test more individuals and more loci.

Novel mutations in *SPINK5* encoding a serine-protease inhibitor in Netherton syndrome, a severe congenital ichthyosis with hair abnormalities and atopic manifestations. *E. Bitoun*¹, *S. Chavanas*¹, *A.D. Irvine*², *M. Paradisi*³, *C. Bodemer*⁴, *D. Hamel-Teillac*⁴, *L. Lonie*¹, *S-i. Ansai*⁵, *Y. Mitsuhashi*⁵, *M. Zimmer*¹, *Y. de Prost*⁴, *G. Zambruno*³, *J.I. Harper*², *A. Hovnanian*¹. 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; 2) Great Ormond Street Hospital, London, UK; 3) Immacolata Hospital, Rome, Italy; 4) Necker Hospital, Paris, France; 5) Yamagata Hospital, Yamagata, Japan.

Netherton Syndrome (NS) is a rare autosomal recessive disorder characterized by erythrodermic ichthyosis, "bamboo-hair", and atopic manifestations. We recently demonstrated that mutations in *SPINK5* (Serine Protease Inhibitor, Kazal-Type 5) cause NS with no evidence for locus heterogeneity (Chavanas *et al.*, 2000, *Nature Genet.*, 25:141-142). Here we screened 24 new families originating from Europe, Middle-East and Asia for *SPINK5* mutations using Denaturing High-Performance Liquid Chromatography (DHPLC). Parents were known to be consanguineous in 4 families. All 27 patients presented with generalized scaly erythroderma, which converted to ichthyosis linear circumflexa in 10 patients between 2 months and 6 years of age. All patients exhibited eczematous rashes and high IgE levels often in response to food allergens. Hypereosinophilia (n=6), hayfever (n=7) and angio-oedema (n=8) were frequently associated with NS. DHPLC analysis of 17/33 exons of *SPINK5* led to the identification of 13 mutations, of which 11 are novel mutations. All mutations predict the formation of premature termination codons. These mutations include four nonsense, four frameshift and five splice site mutations in exons 1, 4, 13, 24-26 and introns 2 and 17. Four mutations were found to be recurrent, each of them occurring in two families not known to be related. Northern blot analysis showed normal levels of *SPINK5* mRNA in some patients, suggesting the synthesis of a truncated protein. We also identified 41 SNPs in patients and controls. Twenty of these SNPs were exonic, 11 of which were non conservative changes. This study provides further evidence for genetic heterogeneity in *SPINK5* mutations in NS and suggests that regulation of proteolysis plays a key role in skin barrier formation and immunity.

Disruption of desmoplakin/intermediate filament interactions results in keratoderma, woolly hair and cardiomyopathy. *S.J. Hatsell¹, E.E. Norgett¹, J.E.A. Common¹, J-C. Ruiz Cabezas², L. Carvajal-Huerta², H.P.*

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Palmoplantar keratodermas (PPKs) are a heterogeneous group of skin diseases which primarily affect the palms and soles or palmoplantar skin. PPKs can be divided, by the pattern of keratoderma, into three main groups, the diffuse, focal and punctate PPK. PPKs can occur as both non-syndromic and syndromic disorders. In this study we have investigated a syndromic form of keratoderma. Three families from Ecuador have recessive striate PPK, a form of focal PPK, which also affects other body sites subjected to stress. Affected areas show longitudinal stripes of hyperkeratosis. The PPK cosegregates with woolly hair and left ventricular dilated cardiomyopathy, often resulting in heart failure and death in early teens. A genome scan revealed affected individuals were homozygous for the same alleles for microsatellite markers mapping to 6p24-25, where desmoplakin also maps. Desmoplakin is the most abundant protein in desmosomes, which are responsible for adhesion between cells and attachment of the intermediate filament network to the cell membrane in most epithelium. The gene encoding desmoplakin was investigated using both dHPLC and sequencing. A homozygous single base deletion was found in exon 24 near to the C terminal. The deletion introduces a stop codon 55 bases downstream resulting in the loss of the C domain of the tail region. To investigate the consequences of the loss of the C domain, which is implicated in intermediate filament binding, immunohistochemistry, confocal and electron microscopy was performed on skin, hair, heart tissue and culture cells from affected family members. A deleterious affect was seen, for example in the skin, with abnormal distribution of desmoplakin and keratin filament collapse. This study provides further evidence for the importance of desmoplakin not only in the skin, but also in maintaining the structural integrity of cardiac tissue.

No evidence for *PTEN* mutations as a common cause of Sotos syndrome or autosomal dominant macrocephaly. S.

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Sotos syndrome is an overgrowth disorder characterised by a typical childhood facial appearance, which includes macrocephaly, frontal bossing, a prominent jaw and a sparse hairline. Additional features include developmental delay, advanced bone age, excessive height in children, early hypotonia and a possible increased risk of cancer. Sotos syndrome has many phenotypic similarities with Bannayan-Riley-Ruvalcaba (BRR), particularly in young childhood, before the characteristic penile freckling and lipomas of BRR become apparent. Germline alterations of the *PTEN* gene are detected in 60% of BRR cases. We are analysing samples from 33 patients with classical Sotos, 18 patients with a phenotype similar to Sotos, (but not the classical features) and 5 families with autosomal dominant macrocephaly, for germline mutations in *PTEN*. The nine exons and intron/exon junctions of *PTEN* are being screened by DNA heteroduplex/SSCP analysis in eleven fragments. We have thus far excluded pathogenic *PTEN* mutations in >90% of the samples. These results indicate that *PTEN* mutations are highly unlikely to be a common cause of either Sotos syndrome or autosomal dominant macrocephaly.

OTX2 gene mutation screening in a large sample of patients with idiopathic hypogonadotropic hypogonadism.

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The protein product from the Otx2 gene appears to be involved in brain morphogenesis in vertebrates, including centers governing reproduction. There is evidence that Otx2 is expressed in gonadotropin releasing hormone (GnRH) neurons in vivo in the mouse and regulates GnRH gene expression in the rat. Humans with idiopathic hypogonadotropic hypogonadism (IHH) have GnRH deficiency, manifested by irreversible pubertal delay, low serum gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH), and sterility. Since the genetic basis for most cases of IHH remains unknown, the human OTX2 gene becomes a logical candidate gene for mutations in these patients. In order to determine if OTX2 gene mutations are present, 125 IHH patients and 25 controls were studied by denaturing gradient gel electrophoresis (DGGE) of GC-clamped PCR products. DNA was extracted, then subjected to polymerase chain reaction (PCR) using a primer pair, one of which contained a GC-clamp. Four fragments spanning the protein coding regions of all three exons were analyzed. The PCR products were first electrophoresed on agarose gels to document the presence of the fragment. The PCR products were then electrophoresed at 60 degrees C in 6.5 % polyacrylamide denaturing gradient gels with 20-80% denaturants (100% denaturants consisted of 40% formamide and 7 M urea). No homozygous deletions were identified in any patient or control, however several variant fragments were identified in IHH patients, which are currently being sequenced. If these DNA sequence differences represent true mutations in IHH patients, the OTX2 gene will be confirmed to play a role in human pubertal development and reproduction. (L.C.L. was supported by USPHS-NICHD HD33004).

Leber Congenital Amaurosis with Anterior Keratoconus in Pakistani families is caused by the Trp278X

Mutation in the AIPL1 Gene on 17p. *K.F. Damji¹, M.M. Sohocki³, R. Khan², S.K. Gupta¹, M. Rahim², M. Loyer⁴, N. Hussein⁴, N. Karim⁵, S.S. Ladak⁵, A. Jamal⁵, D. Bulman¹, R.K. Koenekoop⁴.* 1) Ottawa Hospital Research Institute, Ottawa, ON; 2) Aga Khan Health Services, Pakistan; 3) Human Genetics Center, Houston, TX; 4) McGill University, Montreal, QC; 5) N/A.

PURPOSE: Leber Congenital Amaurosis (LCA) represents the earliest and severest retinal dystrophy leading to congenital blindness. It has a multigenic basis and is proving central to our understanding of the development of the retina. We describe here the clinical and molecular genetic features of Northern Pakistani pedigrees with keratoconus in some affected individuals with LCA. **METHODS:** Histories, physical exams, and eye examinations were performed in the field. Venipunctures, DNA extractions, linkage studies to known LCA genes, automated sequencing, and polymorphism analyses for haplotype assessments were also done. **RESULTS:** Four pedigrees were identified with a severe and variable form of LCA. Affecteds had hand motion to no light perception vision, presence or absence of keratoconus, and retinas ranging from a maculopathy to diffuse pigmentary retinopathy. In each family, we found a homozygous nonsense mutation in the AIPL1 gene, which replaces a tryptophan with a stop codon (Trp278X). Based on 3 internal polymorphisms in AIPL1, the haplotype sequence of our four pedigrees was found to be GCG, indicating that they are likely derived from a recent common ancestor. **CONCLUSIONS:** We identified 4 Northern Pakistani families with a severe, heterogeneous form of LCA that is associated with severe keratoconus in some affecteds. The molecular etiology in all 4 families is a homozygous, nonsense mutation, Trp278X in the photoreceptor-pineal gene AIPL1. To our knowledge, this is the first detailed phenotype-genotype correlation of AIPL1-associated LCA. This study provides the family members and ophthalmologists with a powerful tool for premarital carrier status assessment, as well as prenatal diagnosis, and adds to our global attempt to classify this important disease at the molecular level and understand basic retinal physiology and pathology.

Implications from mutational screening of the cationic trypsinogen gene in a large cohort of subjects with idiopathic chronic pancreatitis. *O. Raguenes¹, A. Piepoli Bis², L. Le Bodic³, P. Ruszniewski⁴, M. Robaszkiewicz¹, P.H. Deprez⁵, Q. Isabelle¹, A. Andriulli², J.M. Chen¹, C. Ferec¹.* 1) Centre de Biogenetique, CHU, UBO, EFS-Bretagne, Brest, France; 2) Divisione di Gastroenterologia, Ospedale "Casa Sollievo della Sofferenza", Istituto di Ricovero e Cura a Carattere Scientifico, San Giovanni Rotondo, Italy; 3) Clinique des Maladies de l'Appareil Digestif, Hopital Laennec, Nantes, France; 4) Service de Gastroenterologie, Hopital Beaujon, Clichy, France; 5) Service de Gastro-Enterologie, Cliniques Universitaires St-Luc, Universite Catholique de Louvain, Brussels, Belgium.

Background: Two missense mutations in the cationic trypsinogen (CT) gene, R122H (R117H) and N29I (N21I), have been clearly associated with hereditary pancreatitis (HP). However, reports regarding their occurrence in the sporadic disease, and whether additional mutations in the gene predispose someone to pancreatitis, are confusing. These controversial issues, in turn, led to controversial attitudes towards whether and how genetic testing of CT mutations should be performed in the sporadic disease. **Methods:** We addressed these issues through mutational screening of the entire coding sequence and the intronic/exonic boundaries of the CT gene in 221 patients with idiopathic chronic pancreatitis, using a previously established denaturing gradient gel electrophoresis technique. **Results:** Of the two HP-causing mutations, only the R122H was detected, and only in a single subject. Additional missense mutations, including A16V, P36R, E79K, G83E, K92N and V123M, were identified once or, at most, twice in separate cases. **Discussion:** These results clearly indicate that the occurrence of the HP-associated CT mutations is detectable but rare in the sporadic disease. Possible predisposition to pancreatitis by some of the additional missense mutations, particularly the A16V signal peptide cleavage site mutation and the V123M substitution, is suspected, but not proven. More extensive association studies are needed to resolve this issue. **Conclusions:** While genetic testing of the R122H and N29I mutations in the sporadic disease is useful, the additional CT missense mutations are only recommended for research purpose.

An inducible adenoviral model of OPMD demonstrates time dependent formation of intranuclear inclusions and apoptosis. *H. Lavoie¹, M-J. Dicaire¹, S. Denis¹, G.A. Rouleau², B. Massie³, Y. Langelier¹, B. Brais¹.* 1) Neurogenetics, CRCHUM, Notre-Dame Hospital, Montreal, Quebec, Canada; 2) Montreal General Hospital, McGill University, Montreal, Quebec, Canada; 3) National Research Council (NRC) of Canada, Biotechnology Research Institute (BRI), Montreal, Quebec, Canada.

Oculopharyngeal muscular dystrophy (OPMD) is an adult-onset disease characterized by progressive eyelid drooping, swallowing difficulties and proximal limb weakness. The autosomal dominant form is caused by short (GCG)8-13 expansions of a (GCG)6 repeat in the poly(A) binding protein nuclear 1 (PABPN1) gene. These mutations lead to the expansion of a polyalanine tract at the N-terminus of PABPN1, a 50 kDa protein involved in the regulation of poly(A) tail length of mRNAs. Objectives: The aim of this study was to establish if the over expression of the mutated protein cause formation of intranuclear inclusions and the apoptosis of eucaryotic cells in a time dependent fashion. Methods: Tetracyclin-inducible adenovirus vectors expressing normal PABPN1 (10 alanines) and mutated PABPN1 (13 alanines) were constructed. Infected A549 tTA cells were studied over 120 hours. Cell death was assessed by apoptosis counts, Hoescht 33342 labelling and TUNEL assay. The expression levels of both wild type and mutant PABPN1 were assessed by western blot. Results: The expression of both wild type and mutant protein cause time dependent apoptosis. However, mutated PABPN1 cause 65% of apoptosis after 120 hours whereas only 35% are apoptotic with the wild type construct. Intranuclear inclusions, a pathological hallmark of the disease, are formed within 12 hours following infection with the mutated construct. They contain PABPN1, proteasomal subunits and ubiquitin. Conclusions: The over expression of mutated PABPN1 cause cell death in a time dependent fashion and the early formation of intranuclear inclusions. This adenoviral model of OPMD may shed some light on the pathophysiology of other triplet repeat diseases with intranuclear inclusions.

Two mutations in the Na⁺-channel alpha-1 subunit gene SCN1A are associated with GEFS+ type 2. *S. Baulac¹, A. Escayg², B. Moulard⁵, I. An-Gourfinkel^{3,4}, D. Chaigne⁵, C. Buresi⁵, B.T. MacDonald², G. Huberfeld¹, A. Brice¹, A. Malafosse⁵, M.H. Meisler², E. LeGuern¹.* 1) Hopital de la Salpetriere, Inserm U289, Paris, France; 2) Department of Human Genetics, University of Michigan, Ann Arbor, Michigan, USA; 3) Centre d'Epilepsie, Hopital de la Salpetriere, Paris, France; 4) Genethon, Evry, France; 5) Division de Neuropsychiatrie, Hopitaux Universitaires de Geneve, Chene-Bourg, Switzerland.

Generalized Epilepsy With Febrile Seizures Plus Type 2 is a dominantly inherited disorder combining febrile seizures, generalized seizures often precipitated by fever after the age of 6 , afebrile generalized seizures and partial seizures. The neuronal voltage-gated sodium channel SCN1A is located on human chromosome 2q24 within the candidate region for GEFS+ Type 2. We describe two mutations of SCN1A , Thr875Met and Arg1648His, that co-segregate with the disorder in two GEFS+ families. Both mutations are located in positively-charged S4 transmembrane segments that determine the voltage-sensitivity of the channel. The residues Thr875 and Arg1648 are evolutionarily conserved in the other mammalian voltage-gated sodium channels and in channels from lower vertebrates and invertebrates. These SCN1A mutations can account for the seizure disorder in GEFS+ and identify a new molecular mechanism for human inherited epilepsy.

Identification of a Fourth Rieger Syndrome Locus at 16q24. *D.Y. Nishimura¹, C.C. Searby², A.S. Borges³, J.C.E. Carani³, A.J. Betinjane³, E.M. Stone⁴, R. Susanna³, W.L.M. Alward⁴, V.C. Sheffield^{1,2}.* 1) Department of Pediatrics, University of Iowa, Iowa City, IA; 2) Howard Hughes Medical Institute, University of Iowa, Iowa City, IA; 3) Department of Ophthalmology, University of Sao Paulo, Brazil; 4) Department of Ophthalmology, University of Iowa, Iowa City, IA.

Rieger syndrome is an autosomal dominant disorder that is characterized by defects of the anterior chamber of the eye that are often associated with glaucoma. The disease phenotype displays a great deal of variation and has been shown to be genetically heterogeneous. Mutations in the PITX2 gene have been found to be responsible for the RIEG1 locus at 4q25, while the IRID1 locus at 6p25 is the result of defects in the FOXC1 gene. A third locus, RIEG2, has been genetically localized to 13q14 by linkage studies performed on a large Rieger syndrome family. Also, the lack of FOXC1 mutations in a number of 6p25-linked families suggests the possibility of a second disease gene at 6p25. We have identified a large Rieger syndrome pedigree from Brazil that contains at least 11 affected individuals. DNA samples were obtained from all 11 affected family members as well as 11 unaffected first-degree relatives. Genotyping of genetic markers from 4q25, 6p25 and 13q14 failed to show evidence of linkage to any of these regions. The Foxc2 knockout mouse has been observed to have eye defects similar to those observed in the Foxc1 knockout mouse. In addition, the conserved forkhead domain is virtually identical between these two genes, which suggests that they might act upon similar targets. Linkage analysis of data from genetic markers near the FOXC2 gene at 16q24 showed that this region was completely linked to the disease within this family. However, a mutation screen of the FOXC2 gene in this family failed to reveal mutations within the coding portion of the gene. In addition, the coding region of FOXC2 has also been examined in 31 probands with Rieger syndrome without detecting any pathological variants. (This work was supported in part by a grant from the Glaucoma Research Foundation.).

Submicroscopic deletion encompassing the *PAX9* gene associated with oligodontia . *P. Das*¹, *D.W. Stockton*^{2,3}, *L.G. Shaffer*², *R. D'Souza*⁴, *T. Wright*⁵, *P.I. Patel*^{1,2}. 1) Department of Neurology; 2) Department of Molecular and Human Genetics; 3) Department of Medicine, Baylor College of Medicine, Houston, TX; 4) Department of Orthodontics, University of Texas-Houston Health Science Center Dental Branch, Houston, TX; 5) University of North Carolina, Chapel Hill.

We have recently identified a frame-shift mutation in the *PAX9* gene as the underlying cause for oligodontia involving posterior teeth (*Nature Genetics*, 24: 18-19, 2000). This mutation was found to segregate in an autosomal dominant pattern in a large North American family. This was the first report on association of a human disorder with the *PAX9* locus. Here we report a small nuclear family in which the father and a child are affected with a similar form of oligodontia affecting both primary and permanent dentitions. An informative single nucleotide polymorphism (SNP) initially suggested hemizyosity at the *PAX9* locus in the two affected individuals. Fluorescence in situ hybridization (FISH) analysis with a cosmid containing the *PAX9* gene confirmed the presence of a deletion involving the *PAX9* locus. To further define the deletion, genotypes were determined at three CA repeat loci in the vicinity of *PAX9* gene. In addition, FISH analysis was conducted with a BAC clone containing a 200 Kb insert. A signal was obtained on both chromosome 14 homologues indicating that the deletion is <200 Kb. We are currently defining the breakpoints of the deletion. These studies support the model of haploinsufficiency for *PAX9* as the underlying basis for oligodontia involving posterior teeth.

Functional implications of GLI3 mutations detected in human polydactylies*GLI3. M. Wessling, M. Kalff-Suske, D. Bornholdt, H. Engel, K.-H. Grzeschik.* Center of Human Genetics, Philipps Universitaet, Marburg.

The GLI3 transcription factor is an important effector protein in the Hedgehog signalling pathway translating positional information during development. Mutations in the human GLI3 gene have been associated with polydactyly syndromes: Greig cephalopolysyndactyly syndrome, Pallister-Hall syndrome, preaxial polydactyly type IV, and postaxial polydactyly type A. Digital anomalies of these syndromes comprise pre-, post-, or central polydactylies as well as syndactylies. So far no obvious association between the phenotype and mutation of specific functional domains of GLI3 is evident. To understand why mutations scattered throughout over 5.0 kb of coding sequence result in different human developmental disorders, we follow a 3-step approach: (i) Extension of the number of mutations identified, in order to gain a reliable insight into the mutational spectrum associated with a single phenotype. (ii) Development of experimental tools and assays to study the following functional properties of GLI3: transcription and stability of mRNA, posttranslational processing, subcellular compartmentalization, DNA- binding, activation/repression of target genes. (iii) Comparison of wild type and mutant protein activities at the following levels: The specificity and affinity of DNA-binding site recognition are determined for bacterially expressed zinc finger peptides and for GLI3 proteins in cell extracts. The capacities for transcriptional activation and repression activities are tested using either fusion proteins with a heterologous DNA-binding domain, or in GLI3/GLI1 competition experiments. Antibodies directed against the amino- and carboxy-termini of GLI3 are used to elucidate the role of phosphorylation-dependent posttranslational processing of endogenous GLI3 or GLI3-GFP fusion proteins. Subcellular compartmentalization of full-length and mutant GLI3 expression constructs is monitored in cultured cells by fluorescence microscopy, and in cell fractions by Western blotting. Understanding the consequences of specific GLI3 mutations for protein function sheds light on the role of individual domains detemining correct anterior/posterior limb development. DFG Gr373/20-3.

SLC7A9 as the main non-Type I Cystinuria gene. *M. Font-Llitjós, The Cystinuria Consortium*^{1,2,3,4,5}. 1) Med. and Mol. Genetics Center, IRO, Hosp. Llobregat, Barcelona, Spain; 2) Dpt. Medicine, Sheba Medical Center, Tel Hashomer, Israel; 3) SGM, Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; 4) TIGEM, San Raffaele Biomedical Science Park, Italy; 5) Dpt. Bioquímica i Biologia Molecular, Fac. Biologia, Universitat de Barcelona, Spain.

Cystinuria (OMIM220100) is a common recessive disorder of renal reabsorption of cystine and dibasic amino acids that results in nephrolithiasis of cystine. Mutations in SLC3A1, which encodes rBAT, cause Type I cystinuria, and mutations in SLC7A9, which encodes a putative subunit of rBAT (b^{0,+}AT), cause non-Type I cystinuria. We established the genomic structure of SLC7A9 and analysed its complete open reading frame in 132 patients from Spain, Italy, North America and Libian jews population. Thirty new mutations were found in this gene: 9 frameshift (7 deletions and 2 insertions), 1 nonsense mutation, 4 splice site mutations and 23 mutations affecting single amino acid residues. These mutations together with the seven previously reported explain 78% of the non-Type I alleles and 44% of the alleles of the mixed-Type (Type I/non-Type I) patients. These data demonstrate that SLC7A9 is the main non-Type I cystinuria gene.

Precise genetic mapping and haplotype analysis of the Mucopolidosis IV candidate region on human chromosome

19p13.2-13.3. *J.S. Acierno Jr¹, J. Falardeau¹, J.C. Kennedy¹, M. Leyne¹, M.C. Bromley¹, M. Colman¹, M. Sun³, C. Bove², G. Bach⁴, L. Ashworth⁵, T. Schiripo¹, S. Ma¹, L.A. Helbling¹, E. Goldin³, R. Schiffmann³, S.A. Slaugenhaupt¹.* 1) Harvard Institute of Human Genetics, Harvard Medical School, Boston, MA; 2) Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown, MA; 3) Developmental and Metabolic Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MA; 4) Department of Human Genetics, Hadassah University Hospital, Jerusalem, Israel; 5) Human Genome Center, Lawrence Livermore National Laboratory, Livermore, CA.

Mucopolidosis IV (ML4) is a rare autosomal recessive lysosomal storage disorder characterized by corneal clouding, abnormal systemic storage bodies, delayed motor milestones, and mental retardation. To date, more than 80 ML4 patients have been reported, the majority being Ashkenazi Jewish (AJ).

We previously had localized the ML4 gene through linkage and haplotype analysis to a 1.2cM region on chromosome 19p13.2-13.3 between markers D19S406 and D19S912, and constructed a 1.4Mb physical map of BAC's and cosmids spanning this region. Since our last report, we have identified nine new polymorphic markers which have allowed us to refine our haplotype analysis. Currently, we have identified 2 haplotypes that account for greater than 97% of the AJ disease chromosomes. Two additional AJ chromosomes are unique, but always coupled with one of the major haplotypes. Haplotype analysis of the major AJ chromosomes demonstrates ancestral recombination events that allow us to narrow our critical region to approximately 170kb. Within this region we have used a combination of EST mapping, exon trapping, and gene prediction to assemble 13 genes for mutation screening--a number of which are novel transcripts. Additionally, we report for the first time the haplotypes of non-AJ patients. Complementation studies show these patients carry mutations in the same gene as the AJ patients, resulting in the possibility of 2 major and up to 8 independent minor mutation events that account for ML4.

Localization of a novel form of juvenile rheumatoid arthritis (familial recurrent arthritis) to human chromosome

15q. *J.D. Gillum¹, L.B. Bennett², V. Pascual³, A.M. Bowcock², M. Lovett², C.A. Wise¹.* 1) Research, Texas Scottish Rite Hosp., Dallas, TX; 2) Washington University School of Medicine, St., Louis, MO; 3) University of Texas Southwestern Medical Center and Baylor Institute for Immunology Research, Dallas, TX.

Most human autoimmune disorders arise from complex genetic and environmental interactions. One such disease is juvenile idiopathic arthritis (JIA), also known as juvenile rheumatoid arthritis (JRA), an autoimmune disorder comprising a heterogeneous collection of chronic arthritides of childhood. This disease is common in the pediatric population, affecting 1-3 children per 1000. It is generally accepted that JIA encompasses several distinct, yet possibly related, clinical entities that exhibit complex inheritance patterns. We ascertained a three generation family in which nine members were diagnosed with JIA. In this family the disease was of very early onset and included episodic inflammation leading to eventual destruction of joints, muscle, and skin. The disorder in this family was treated as a distinct clinical entity that we have named "familial recurrent arthritis" (FRA). A genome-wide linkage scan localized the causative gene to a ~20 cM interval on chromosome 15q, encompassing the region recently shown to be linked to PAPA (pyogenic sterile arthritis, pyoderma gangrenosum, and severe cystic acne) syndrome. PAPA syndrome, described in a single extended kindred, is a fully penetrant autosomal dominant trait marked by acute, steroid-responsive inflammations and skin lesions. Given their clinical similarities and linkage to the same critical interval, we hypothesize that FRA and PAPA syndrome are the same disorder. From searches of public databases we have assembled candidate genes and ESTs from the FRA interval. The interleukin 16 (IL16) gene maps to the interval and is a plausible candidate. However, direct DNA sequencing of the entire coding and promoter region has failed to identify any mutations. Mutation screening of additional positional candidates is in progress. Identification of the FRA gene and subsequent functional analyses will provide important insights into the causes of autoimmune destruction.

Mutations in the nonmuscle myosin heavy chain IIA gene (*MYH9*) result in the diverse phenotypes of the May-Hegglin anomaly, Fechtner and Sebastian syndromes. *International Hereditary Macrothrombocytopenia*

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The autosomal dominant giant platelet disorders, May-Hegglin anomaly (MHA), Fechtner syndrome (FTNS) and Sebastian syndrome (SBS), share the clinical triad of thrombocytopenia, large platelets, and characteristic leukocyte inclusions ('Döhle-like' bodies). While MHA and SBS can be differentiated by subtle ultrastructural features of their leukocyte inclusions, FTNS is easily distinguished by the additional clinical features of sensorineural deafness, cataracts, and nephritis. The similarities between these platelet disorders and our recent refinement of the MHA and FTNS disease loci to an overlapping region of 480kb on chromosome 22 suggested that all three disorders are allelic. Among the candidate genes in this region was the nonmuscle myosin heavy chain 9 gene (*MYH9*); expressed in platelets and up-regulated during granulocyte differentiation. Six *MYH9* mutations (one nonsense and five missense) were identified in seven unrelated probands from MHA, SBS, and FTNS families. Of particular interest, and based on crystallographic structure modelling, the two myosin head mutations were predicted to impose electrostatic and conformational changes while the truncating mutation deleted the unique C-terminal tailpiece. The remaining missense mutations, all within highly conserved positions, imparted destabilizing electrostatic and polar changes within the coiled-coil domain. Thus, our results suggest that mutations in nonmuscle myosin IIA result in these three megakaryocyte/platelet/leukocyte syndromes and are important in the pathogenesis of sensorineural deafness, cataracts, and nephritis.

Analysis of candidate genes in the CFM1 region on chromosome 19q13. *J.S. Zielenski, D. Markiewicz, M. Corey, I. Aznarez, R. Rozmahel, P. Durie, L.-C. Tsui and the CF Modifier Collaborative Group.* Dept Genetics, Hosp Sick Children, Toronto, ON., Canada.

Cystic fibrosis is primarily caused by mutations in the CFTR gene but the CF phenotype is also influenced by secondary genetic modifiers. We previously demonstrated the presence of a CF modifier locus (CFM1) on human chromosome 19, region q13.2. CFM1 contributes to the predisposition of meconium ileus (MI). To narrow the critical region for CFM1, we have performed transmission disequilibrium test (TDT) for markers in the above interval. The study population included 220 "trios" (CF patients with MI and their parents). To date, we have tested 16 STR or SNP markers: D19S408-40867/DraI-D19S574-APOC2-APOC1/HpaI-2050/AvaII-31237(GT)n-10080(GT)n-D19S219-DM(GCT)n-D19S112-D19S412-D19S902-D19S604-D19S246-KLK1. The only marker that showed a notable excess of transmission of one allele is the STR in the APOC2 gene on the basis of 124 informative trios (%T=63% $c^2=5.00$; df=1; $P<0.02$). In addition, four genes in the D19S408-D19S412 region, namely, CALM3, STD, PEREC1 and KCNN4 have been analyzed as possible candidates on the basis of the functional characteristic of the encoded proteins, their apparent relevance to MI and position in relation to the CFM1 region. The promoter and coding regions of these genes were analyzed by direct DNA sequencing of several CF sibpairs discordant for MI. Twenty-seven nucleotide sequence variants have been detected in the analyzed genes but no apparent potential mutations were found. Case-control and/or TDT analysis of selected intragenic markers from each gene were performed in MI individuals or MI trios to assess their allelic association with MI. The most intriguing observation is the allelic association detected for the intragenic STR D19S217 marker in intron 1 of the KCNN4 gene with excess transmission of one of the most abundant alleles (%T=62% $c^2=7.7$; df=1; $P = 0.0055$). The significance of this result is being further investigated. The CF Modifier Collaborative Group: T.Casals, C. Castellani, D. Bozon, M. Claustres, B. Marshall, E. Bjorck, A. Palacio, E. Langfelder, J. Bal, G.Cutting, L.Chertkoff, C. Ferec, B. Strandvik, M. Macek Jr., X. Estivill.

Characterization of additional cochlear ESTs for gene discovery in the auditory system. *B.L. Resendes^{1,2}, N.G. Robertson¹, J.D. Szustakowski³, R.J. Resendes⁴, Z. Weng³, C.C. Morton^{1,2}*. 1) Dept OB/GYN, Thorn Bldg, Brigham & Women's Hosp, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Dept. Biomedical Engineering, Boston University, Boston, MA; 4) Sun Microsystems, Inc., Burlington, MA.

To identify auditory system genes, 8494 ESTs were generated from a human fetal cochlear cDNA library in two sequencing projects. Analysis of the first set of ESTs (n = 4304), described previously, revealed 517 known human genes, 40 nonhuman mammalian genes, 487 ESTs from other libraries, and 541 cochlear specific ESTs (<http://hearing.bwh.harvard.edu>).

We now report results of a BLAST analysis of the additional cochlear ESTs (n=4190) and a comparison to the first 4304 ESTs. Among the 4190 new cochlear ESTs, 808 represent 631 additional known human genes, 73 represent 65 additional known nonhuman mammalian genes, 1357 represent 1230 additional ESTs found in other libraries, and 262 of the new ESTs do not identify any other ESTs and are considered cochlear specific. Map positions are known for 404 of the ESTs that did not match a known gene and revealed additional loci for every chromosome. We performed a comparative analysis of the ESTs to determine redundancy of the two efforts. 1690 ESTs were found in common, including 246 known human genes identified in the original analysis. Reanalysis of ESTs from the prior study that had not matched known genes revealed an additional 20 genes.

The total number of known human genes identified from the 8494 cochlear ESTs is 1153 and is represented by 3034 ESTs (36.77%). Of the 1153 human genes, 522 (45.3%) genes, represented by 1388 ESTs, were identified previously and 631 (54.7%) genes, represented by 808 ESTs, were identified from the most recent sequencing effort. The total number of ESTs that do not identify known genes is 5090 (61.68%). Over 800 of these do not match other ESTs and may represent unique or preferentially expressed cochlear genes. Identification of additional known genes and cochlear-specific ESTs, provides new candidate genes for both syndromic and nonsyndromic deafness disorders.

Fine-mapping of Naegeli-Franceschetti-Jadassohn syndrome to 7 cM in 17q11.2-q21, and exclusion of 18

positional candidate genes. *E. Sprecher*^{1,2}, *P. Itin*³, *N. Whittock*⁴, *J. McGrath*⁴, *J. Uitto*², *G. Richard*². 1)

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Naegeli-Franceschetti-Jadassohn syndrome (NFJS) is a rare, autosomal dominant ectodermal dysplasia characterized by a congenital absence of dermatoglyphics, reticulate hyperpigmentation of the skin, impaired sweating, and variable hair and enamel abnormalities. NFJS was recently mapped to a 33cM region on 17q (Whittock et al., J Invest Dermatol 114:827, 2000). We ascertained and re-examined 22 descendants of the original Swiss family, including 9 affected individuals in 3 successive generations. Using automated genotyping, we determined the genotype of each family member for 12 polymorphic microsatellite markers spanning 45cM, and constructed a parsimonious haplotype for the family. Multipoint linkage analysis revealed a maximum LOD score of 2.7 at D17S800, which is supportive of linkage. Using haplotype analysis, we were able to substantially narrow the disease gene interval to about 7cM in 17q11.2-q21 flanked by D17S933 and D17S1860. This region harbors the type I keratin gene cluster, and contains more than 45 positional candidate genes. Initially, we excluded several genes known to be expressed in ectodermal tissues by screening the patients DNA for pathogenic mutations using direct DNA sequencing (KRT15, KRT19, KRT20, DLX3, DLX4, RARA, and GRN). We then established the genomic structure of 11 additional candidates, but failed to identify pathogenic mutations in these genes. Among them were 2 cDNAs encoding hitherto unknown proteins with high sequence homology to type I keratins. DNA sequence and expression of the remaining positional candidate genes are currently being scrutinized to eventually identify the NFJS gene.

Potential role of *NODAL* and *CRIPTO* in holoprosencephaly. *J.M dela Cruz, R.N. Bamford, E. Roessler, M. Muenke.* Medical Genetics Branch, NHGRI/NIH, Bethesda, MD.

At the onset of vertebrate gastrulation, the TGF- β -related ligand Nodal is expressed in dorsal mesoderm progenitors and, subsequently, in the left lateral plate mesoderm. From these cellular positions, Nodal signaling controls mesoderm formation, left-right and anterior-posterior axis positioning, and neural patterning. Based on animal models, defects in this pathway are likely to contribute to human disease, including anterior midline abnormalities and laterality defects. Nodal mediates its signaling through Activin-like receptors, Smad2/Smad4 and FAST proteins, ultimately driving the transcription of many downstream targets. Additionally, Nodal is tightly regulated at many levels by several different factors, including the extracellular EGF-CFC protein Cripto that by an unknown mechanism acts as an obligate cofactor for Nodal. Mice made null for *nodal*^{-/-} by targeted gene disruption never form a primitive streak, lack most mesoderm, and die in early development. However, when mice heterozygous for *nodal*^{+/-} are bred with mice heterozygous for *smad2*^{+/-}, many of the resulting trans-heterozygotes survive gestation but succumb early to severe anterior midline and laterality defects. More specifically, 54% of these animals demonstrate craniofacial anomalies including cyclopia that is very reminiscent of human holoprosencephaly (HPE). Importantly, mice null for *cripto*^{-/-} have a phenotype very similar to the *nodal*^{-/-} mice, emphasizing Cripto's obligate role in the Nodal-signaling pathway. Noting this and the HPE-like phenotype of the *nodal*^{+/-};*smad2*^{+/-} mice, all of these genes are good candidates of human HPE. Currently, *CRIPTO* and *NODAL* are being evaluated for their potential role in HPE by SSCP and denaturing HPLC. DNA from patients with sporadic HPE have demonstrated two missense mutations in the *CRIPTO* gene. Normal control assays have been performed on each mutation that demonstrated the absence of these nucleotide changes in at least 130 normal chromosomes. Mutational analysis of *NODAL* on familial genomic DNA has been performed yielding negative results; however, *NODAL*'s role in human sporadic HPE will be further investigated.

Discovery and genotyping of single nucleotide polymorphisms in candidate genes associated with bipolar disorder. *G. Stevenson¹, J.M. Devaney², M.A. Marino², D.A. Stephan¹.* 1) Research Center for Genetic Medicine, Children's National Medical Center, Washington , DC; 2) Transgenomic, Inc. Gaithersburg, MD.

Bipolar disorder (BPD) is a common disease affecting ~1% of the world's population. This disabling condition involves dysregulation of multiple physiologic functions, including mood, appetite, sleep and activity. BPD has been observed to aggregate within families suggesting a genetic contribution to risk. However, segregation analysis has not been consistent with a single gene mode of inheritance; several genes could act together in a given individual to cause BPD. We have identified several founder populations that would be amenable to genotyping at candidate loci to identify a common haplotype or haplotypes associated with the phenotype.

In an attempt to find disease-causing genes for BPD, we identified 100 genes that encode proteins involved in neuroendocrine physiology, neurotransmission, and central nervous system development. We are identifying single nucleotide polymorphisms (SNPs) in these genes using denaturing high performance liquid chromatography (DHPLC). RNA was extracted from 24 frozen brains from unrelated individuals of varied ethnic origin and reverse transcribed into cDNA. Primers overlapping through the coding regions of the transcripts were amplified and screened directly for SNPs with by DHPLC, under the assumption that any informative SNPs useful for genotyping populations will not be homozygous in the identification phase of the study. We are scanning the candidate genes from patient samples with BPD for SNPs in the hopes of a significant association result using DHPLC coupled with a non-fluorescent multiplexed single-base extension procedure. The SNPs discovered will hopefully allow for associative studies to find a genetic factor for bipolar disorder.

Confirmation of the gene for autosomal medullary cystic kidney disease *ADMCKD2* on chromosome 16p12. *N.*

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Autosomal dominant medullary cystic kidney disease is an interstitial nephropathy characterised by a defect in urinary concentration ability. Two genes predisposing to ADMCKD have been localised. *ADMCKD1* was localised to chromosome 1q21 in two Cypriot families with ADMCKD, hyperuricemia and gout. *ADMCKD2* was localised to chromosome 16p12 in a single Italian family with ADMCKD, hyperuricemia and gout.

We have identified a large Welsh family that fulfills the criteria for ADMCKD. We have evaluated the family for linkage to the two known predisposition genes. Linkage to *ADMCKD1* was excluded (two-point LOD score = -5.3). Analyses at *ADMCKD2* provide strong evidence of linkage (two-point LOD score = 3.0) and refines the interval encompassing the gene to a 10cM interval between D16S764 and D16S420. These data provide independent confirmation for the location of *ADMCKD2*. Unlike previous families, no member of the Welsh family has hyperuricemia or gout, indicating that these are not obligatory features of the condition.

Recently, a gene for familial juvenile hyperuricemic nephropathy (FJHN) was localised to the same interval on chromosome 16p. From evaluation of the clinical and pathological phenotypes of the two conditions, we suggest that *ADMCKD2* and FJHN are likely to be due to mutation of the same gene.

MUTATIONS IN TRANSFERRIN RECEPTOR-2 IN HEMOCHROMATOSIS TYPE 3. A. Roetto¹, C.

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Hemochromatosis is a common recessive disorder which leads to iron overload and severe clinical complications. Most patients are homozygous for C282Y in HFE on 6p. Few patients have a severe form with expression at young age (juvenile hemochromatosis or HFE2). Recently a third form has been described linked to 7q22 (HFE3). Six Italian patients have been reported homozygous for Y250X mutation in transferrin receptor 2 (TFR2) (Camaschella et al, Nat Genet 25:14-15,2000). Here we report the screening for mutations in 9 Italian patients and the identification of a new TFR2 mutation in 4 patients from an unrelated family with a high degree of consanguinity affected by HFE3. Exploiting homozygosity mapping in this family we excluded linkage to 6p and 1q. Using 7q microsatellite markers (D7S647, D7S2480, D7S662, D7S477) and two TFR2 intragenic polymorphic repeats we showed that all patients had an extended homozygosity region at these markers. Scanning TFR2 by direct sequencing a new mutation (88insC) was detected at the homozygous state in exon 2. This insertion causes a frameshift and a premature stop codon at amino acid 60 (E60X). TFR2 is homologous to transferrin receptor, has a still unclear function and shows two alternatively spliced forms, a and b. At variance with Y250X which affects both variants, the newly discovered mutation disrupts only the a variant, leaving the b unaffected. Comparison of the phenotype indicates a more severe phenotype in Y250X homozygotes than in 88insC homozygotes. Our data confirm that TFR2 inactivation causes HFE3 and suggest a modulation of the clinical phenotype according to the mutation position.

Structural organization, transcript expression and mutational analysis of the human Rab geranylgeranyl transferase alpha subunit (*RABGGTA*) gene. W. Li¹, J.C. Detter², H.J. Weiss³, E.M. Cramer⁴, Q. Zhang¹, E.K. Novak¹, R. Favier⁴, S.F. Kingsmore⁵, R.T. Swank¹. 1) Department of Molecular & Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY; 2) DOE Joint Genome Institute, Walnut Creek, CA; 3) Division of Hematology-Oncology, St Lukes-Roosevelt Hospital Center, New York, NY; 4) Departement d'Hematologie ICGM, Hopital Cochin, Maternite Port Royal, Paris, France; 5) Molecular Staging Inc., Guilford, CT.

Hermansky-Pudlak Syndrome (HPS) is a recessively inherited multigenic disease with dysfunction of several related subcellular organelles including platelet dense granules, melanosomes, and lysosomes. Recent findings that one mouse model of HPS, the gunmetal mouse, is associated with abnormal expression of the Rab geranylgeranyl transferase α -subunit gene (*Rabggta*) prompted re-analysis of the structure of its orthologous human gene (*RABGGTA*) in normal individuals and in patients with deficiencies of dense granules (d-SPD), alpha granules (a-SPD, or gray platelet syndrome, GPS), or alpha plus dense granules (ad-SPD). The exon/intron structural organization of the 5' upstream region of human *RABGGTA* was found similar to that of the mouse *Rabggta* gene although the sequences of exons a and b (or intron a) are not homologous. We report the complete structure of intron alpha and suggestive data on transcription start sites. Features of the 5' upstream region (GC-rich, no TATA box, variable transcription start sites) of *RABGGTA* suggest it is a house keeping gene. While obvious disease causing mutations of human *RABGGTA* were not found in our existing patients, several polymorphisms of *RABGGTA* including an introduction of a new restriction enzyme site were identified in selected patients. Knowledge of the 5' structure of *RABGGTA* and its common polymorphisms will be useful for mutation screening or linkage analysis in patients with albinism, thrombocytopenia or platelet SPD. This information will also be useful for genetic analysis of families that may have autosomal recessive congenital ichthyosis (ARCI) caused by abnormalities in the neighboring transglutaminase 1 (TGM1) gene, which is arranged in head-to-tail fashion with *RABGGTA*.

Genomic structure of postsynaptic organizational protein, Gephyrin, and mutation analysis in hyperekplexia and startle phenotypes. *R.G. Snell¹, J. Miller¹, M.J. Owen², M.I. Rees¹*. 1) Department of Molecular Medicine, University of Auckland Medical School, Auckland, New Zealand; 2) Department of Psychological Medicine, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, UK.

The association of α_1 and β -subunit mutations in the inhibitory glycine receptor (GlyR) in only a proportion of startle disease patients (hyperekplexia) indicates the involvement of other genetic determinants that contribute to this neurological disorder. One such candidate is gephyrin, a protein responsible for the anchoring and clustering of GlyRs and GABAA sub-types to the postsynaptic membrane of inhibitory neurones in mammalian brain and spinal cord. Gephyrin has a pleiotropic nature, since evidence suggests a further non-neurological role in the biosynthesis of molybdenum enzymes. Here we describe the genomic structure of human gephyrin isolated by *in silico* cloning from chromosome 14 BAC sequences. Homology searches with the rat gephyrin cDNA revealed that human gephyrin has 22 exons distributed over 670Kb of genomic DNA, including the 5' and 3' UTR sequences. The rat, chicken and human open reading frames are 2211bp in length and have a 94% (rat) and 88% (chicken) sequence homology reflecting a highly conserved 99.7% and 98% amino acid parity respectively. Three of four splicing cassettes present in rat cDNA isoforms are represented in human genomic DNA as discrete exons, suggesting similarities in RNA isoform heterogeneity. Mutation analysis of human gephyrin exons, 3'/5' UTRs and putative promoter region was conducted on a cohort of 30 hyperekplexia patients using SSCP and bi-directional di-deoxy fingerprinting (DDF). Both methods have detected a range of common and rare variant profiles in several exons, which are currently being characterised by sequencing. In summary, we present the human genomic structure of an important neurological gene involved in the orchestration of inhibitory neurotransmission and a candidate gene in both hyperekplexia and familial molybdenum deficiency.

Identification of a deletion in 3q23 causing the Blepharophimosis, Ptosis and Epicantus inversus Syndrome

(BPES). *M.-L. Bondeson¹, K. Lagerstedt¹, G. Anneren¹, S. Jagell², L. Zech¹.* 1) Dept. of Genetics & Pathology, Rudbeck Laboratory, Uppsala University Hospital, Uppsala, Sweden; 2) Dept. of Pediatrics, Gavle County Hospital, Gavle, Sweden.

Blepharophimosis, Ptosis and Epicantus inversus Syndrome (BPES) is a genetic disorder affecting craniofacial development. The primary features of BPES include shortening of the palpebral fissures (blepharophimosis), ptosis of the eyelids, epicanthus inversus and telecanthus. Two types of the syndrome have been suggested where Type I presents with primary ovarian failure and female sex-limited infertility in addition to the facial features. Type II BPES appears to present with the classical features alone. In addition to the classical features a number of associated anomalies have also been described for BPES including cardiac defects, abnormally shaped ears, a broad flat nasal bridge and microcephaly. Most cases of BPES are sporadic, but autosomal dominant inheritance has also been observed. Recent studies of patients with balanced translocations have assigned a BPES critical region to chromosome 3q23. We have identified a boy (3 years of age) with BPES, mental retardation and microcephaly. In addition to the phenotypes normally associated with BPES he has also abnormally shaped ears and a very fair skin. This suggests that the boy is suffering from a contiguous gene deletion syndrome where several genes most likely are affected. Cytogenetic analysis of peripheral blood showed a normal karyotype without detectable deletions or translocations. In order to investigate for sub-microscopic deletions microsatellite analyses with 26 polymorphic markers were performed. Hemizygoty/homozygoty was observed for two of the markers D3S3586 and D3S3554, both of which are located in the BPES critical region. Subsequent FISH analyses with cosmid probes spanning the critical region showed that the boy is suffering from a deletion in 3q23.

Diagnosis of Autosomal Recessive Lamellar Ichthyosis with Mutations in the TGM1 Gene. *P.B. Cserhalmi-Friedman¹, L.M. Milstone³, A.M. Christiano^{1,2}*. 1) Department of Dermatology, Columbia University, New York, NY; 2) Department of Genetics and Development, Columbia University, New York, NY; 3) Department of Dermatology, Yale University, New Haven, CT.

Autosomal Recessive Lamellar Ichthyosis (ARLI) is a clinically and genetically heterogeneous disorder. In many cases, mutations in the transglutaminase 1 gene have been identified, however, other clinically indistinguishable cases have been linked to chromosomes 2, 3 and 19. Previous studies failed to establish any correlation between clinical characteristics and genetic background. We report 10 patients with the typical clinical presentation of lamellar ichthyosis. We performed PCR and direct sequencing based mutation screening in all of these patients, TGM1 immunofluorescence microscopy and in vitro enzyme activity assay in selected patients. Mutation screening revealed 14 mutations, 4 of which have been previously described. While immunofluorescence microscopy was negative in patients with nonsense mutations or out-of-frame insertions or deletions, the results were variable in cases with missense mutations and in cases with no mutation in the TGM1 gene. In vitro enzyme activity assays gave results which were consistent with the mutation data. Our findings support the importance of mutation screening in the evaluation and classification of ARLI.

Spectrum and distribution of MECP2 mutations in 60 Italian Rett Syndrome patients. Tentative genotype/phenotype correlation. *L. Giunti, S. Pelagatti, V. Lazzerini, C. Biondi, S. Guarducci, U. Ricci, E. Lapi, A. Cecconi, L. Ombroni, E. Andreucci, I. Sani, A. Brusaferri, M. Lapini, M.L. Giovannucci Uzielli.* University of Florence, Department of Paediatrics, Genetics and Molecular Medicine Unit, Azienda Meyer, Florence, Italy.

Mutations of methyl-CpG binding protein 2 (MECP2) gene were sought in 60 Italian RTT girls, using the direct DNA sequencing method, with a long-read approach and IR automated fluorescence monolaser sequencer LICOR-4200. Sequencing of the coding region and the splice sites, revealed mutations in 60 of 67 (89.59%) patients, all isolated and unrelated cases, included in this study after a clinical evaluation in our Centre. Some mutations have not been described previously. We report the preliminary results of the genotype/phenotype correlation, based on the clinical data, directly collected in a follow up program, supported by the Italian Association of Rett Syndrome (AIRS). Most mutations (2/3) are truncating: four large, novel deletions of exon 3, two identical in unrelated cases, were also identified. No abnormalities were detected in any of the parents, all available for investigation. Interesting data result from the comparison of the clinical phenotype in groups of RTT subjects with the same MECP2 mutation: factors other than the type and position of MECP2 mutation must be involved in the phenotype expression of this disorder.

Relatedness of SMN gene deletion and conversion with clinical presentation in spinal muscular atrophy. M.Z.

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Spinal muscular atrophy is an autosomal recessive disorder characterized by degeneration of lower motor neurons. We have screened for deletions and a gene conversion event in SMN and NAIP genes using PCR-RFLP methods. Of 108 samples tested, 46 (23 type I, 21 type II and 2 type III) were from SMA patients, and 62 from unaffected controls. SMA cases included 3 from Bahrain, 9 from South Africa, 2 from India, 5 from Oman, 1 from Saudi Arabia and 26 from Kuwait. SMN gene exons 7 and 8 were deleted in all SMA cases and NAIP gene exon 5 and 6 in 22 of 23 type I patients. SMN gene exon 7 was deleted in all and exon 8 in 19 of 21 type II SMA patients. In one type II SMA patient, both centromeric and telomeric copies of SMN exon 8 were deleted. The NAIP gene exon 5 and 6 were deleted only in one type II SMA patient. In one of the two type III cases, SMN exons 7 and 8 were deleted and no deletion was detected in the NAIP gene, while in the second type III patient, deletions were detected in both SMN and NAIP genes. None of the 62 controls had deletions in either SMN or NAIP gene. The presence of a gene conversion event involving SMNt gene and its centromeric copy (SMNc) was also screened in SMA cases and controls. In two type II SMA cases (one from Kuwait and second from South Africa), a chimaeric gene with a fusion of exon 7 of SMNc and exon 8 of the SMNt gene was detected while the normal SMN gene was absent. In contrast to previous studies, we did not find this fusion event in any of our controls and in type I SMA patients.

The spectrum and parental origin of *de novo* mutations of methyl-CpG-binding protein 2 gene (*MECP2*) in Rett syndrome. I. Kondo¹, R. Morishita¹, T. Fukuda¹, K. Obata¹, T. Matsuishi², Y. Yamashita², K. Kuwajima³, I. Horiuchi⁴, I. Omori⁵. 1) Dept. of Hygiene, Ehime Univ. School Medicine, Ehime, Japan; 2) Dept. of Pediatrics and Child Health, Kurume Univ. School of Medicine, Fukuoka, Japan; 3) Dept. of Pediatrics, Ibaraki Handicapped Children's Hospital, Ibaraki, Japan; 4) Asahigawa Jidoin Children's Hospital, Okayama, Japan; 5) Dept. of Child Neurology, Okayama Univ. School of Medicine, Okayama, Japan.

Rett syndrome is a neurodevelopmental disorder characterized by a regression of speech skills and acquired hand use, stereotypical hand movements, autistic behaviors, and seizure after a period of normal early growth. Because RTT affects females exclusively, it has been proposed that it is an X-linked dominant disorder with male lethality, resulting from the *de novo* mutations inherited from the father. Recently, DNA mutations in the methyl-CpG-binding protein 2 gene (*MECP2*) mapped to Xq28 have been identified in patients with RTT. We screened the mutation sites in the *MECP2* in 75 sporadic cases with a clinical diagnosis of RTT and identified 23 different mutations in 69 patients (93.3%), including seven missense mutations (R106W, L124F, R133C, S134C, P152R, T158M, P302H, P302R, R306C), six nonsense mutations (Y141X, R168X, Q170X, R255X, R270X, R294X) and 10 frameshift mutations. In addition, six single nucleotide polymorphisms (SNPs) were found in intron 2 and exon 3 of *MECP2*. Using these SNPs, a parental origin has been elucidated in 26 cases (23 paternal and three maternal), indicative of an exclusive paternal origin. These results may suggest that the predominance of female patients with RTT can be simply explained by sons not inheriting their X chromosome from the father.

Molecular analysis of germinal HPRT mutations in Lesch-Nyhan syndrome and in carrier females. *P. O'Neill, L. Trombley, M. Gundel, J. Nicklas.* Genetics Laboratory, University of Vermont, Burlington, VT.

Lesch-Nyhan syndrome and related diseases are caused by mutations in the X chromosome HPRT gene. The disease occurs almost exclusively in males and females in these families are at risk of being carriers of the mutation. We have developed a method which combines T-lymphocyte culture and DNA sequencing to define the HPRT mutation and the carrier status of females in these families. Most HPRT mutations result in such reduced enzyme activity that cells are able to grow in the presence of the normally cytotoxic purine analogue 6-thioguanine (TG). T-lymphocytes are isolated from peripheral blood samples (heparin) and cultured in vitro in medium containing phytohemagglutinin and interleukin 2 to achieve exponential cell proliferation. Cultures from an affected male grow in the presence of TG and are used as a source for HPRT cDNA sequencing and genomic sequencing to determine the mutation. The frequency of cells in females expressing the HPRT mutant allele is determined through a T-lymphocyte cloning assay, which measures the cell cloning efficiency in the presence and absence of TG. The ratio defines the HPRT mutant frequency. Non-carrier females show a TG resistant mutant frequency of $1-20 \times 10^{-6}$ and carrier females show a frequency of 0.5-10%. When possible, the presence of the mutation in the 'carrier' females is confirmed by genomic sequencing, as well as the absence of the mutation in 'non-carrier' females. We will present results on males from 56 families with 51 different mutations, and on 71 females in these families. In four of these families, the mutations do not allow growth in TG and the males show variant phenotypes. The fundamental reason for this research is to enable females in these families to know if they carry a HPRT mutation. Given the nature of this inherited disease, decisions concerning reproduction can then be made with definitive information about risk. (This research is supported by a grant from the Lesch-Nyhan Syndrome Childrens' Research Foundation.).

Identification of a novel 4.6 KB genomic deletion in presenilin-1 gene resulting in exclusion of exon 9 in a Finnish early onset Alzheimers disease family: an alu core sequence-stimulated recombination? *M.J. Hiltunen^{1,2}, S.*

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Mutations in the presenilin-1 (PSEN-1) gene have been shown to cause early onset Alzheimers disease (EOAD) in an autosomal dominant manner. We have identified a novel 4.6-kb genomic deletion in the PSEN-1 gene in a Finnish EOAD family, which leads to an inframe exclusion of exon 9 (D9) from the mRNA transcript. This germline mutation results in a similar alteration in mRNA level as previously described with the variant AD and the D9 splice-site mutations. In this present EOAD family, the clinical and neuropathological phenotype of patients are those of the typical AD without, according to brain biopsy, indications of spastic paraparesis or cotton wool plaques, which are the hallmarks of the variant AD.

A sequence analysis of the deletion crossover site of the mutant and corresponding wild type regions revealed complete homology with the recombinogenic 26 bp Alu core sequence at the intron 8. In addition, a segment at the intron 9 breakpoint displayed homology with the core sequence, but comparison of the 5 and 3 breakpoint sequences did not reveal significant identity favoring involvement of Alu core sequence-stimulated non-homologous recombination rather than Alu-mediated homologous pairing of the fragments.

This study shows that large genomic rearrangements can affect the EOAD gene PSEN-1 through a mechanism, which may involve Alu core sequence-stimulated recombination.

***SLC7A9* mutations in type II and type III cystinuria.** D. Leclerc¹, M. Boutros¹, D. Suh¹, Q. Wu¹, M. Palacin², P. Goodyer¹, R. Rozen¹. 1) Human Genetics and Pediatrics, McGill Univ., Montreal, Quebec, Canada; 2) Biochemistry and Molecular Biology, Univ. of Barcelona, Barcelona, Spain.

Cystinuria is an inherited disorder of cystine and dibasic amino acid transport in the kidney. The classification of cystinuria subtypes is based on the urinary cystine excretion patterns of obligate heterozygous parents of the probands: Type I (silent, in the normal range); Type II (high excretor); and Type III (moderate excretor). The first gene shown to cause cystinuria (*SLC3A1* or rBAT) is associated with mutations on Type I chromosomes; over 40 mutations have been identified in this gene. Linkage studies suggested that Types II and III cystinuria might be caused by a second gene on chromosome 19q; this gene (*SLC7A9*) was recently isolated and 7 mutations were identified on non-Type I cystinuria alleles. In earlier work, we described novel rBAT mutations in a series of newborn cystinuria patients identified through a Quebec screening program. Virtually all the mutations (in rBAT) were identified in the 8 Type I/I patients. In this report, we describe our mutational analyses of the *SLC7A9* gene in patients with other subtypes. Individual exons of the *SLC7A9* gene were screened by SSCP analysis, followed by sequencing of abnormally-migrating fragments. We have identified 3 novel mutations thus far. One mutation, a single bp insertion at bp799, was identified in 4 patients: one Type I/III patient (mutation linked to Type III allele), 2 with Type II/N (N = normal), and one with Type III/III. A 4bp deletion in intron 12 was identified in a Type I/III patient on the Type III allele, and a nonsense codon (1491 G®T) was identified in a Type I/III patient (allele unknown). Additional mutational analyses are in progress. Our study suggests that Type II and Type III cystinuria can be caused by the same mutation in *SLC7A9* and, therefore, other factors must influence urinary cystine excretion.

Novel COL1A1 mutation (G599C) associated with mild osteogenesis imperfecta and dentinogenesis imperfecta.

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We report a genotype-phenotype analysis of a 3-generation family segregating for an autosomal dominant osteogenesis imperfecta (OI) variant. The family was ascertained through a proband concerned with discoloration of her teeth, determined to be dentinogenesis imperfecta (DGI). Examination of 36 family members identified 15 individuals with DGI. Linkage studies were performed for genetic markers from candidate intervals known to contain genes responsible for DGI on chromosomes 4q, 7q, and 17q. Conclusive evidence for linkage of this DGI syndrome was obtained to genetic markers on chromosome 17q21-q22 (DLX-3, $Z_{\max} = 5.34$, $q = 0.00$). All DGI affected family members shared a common haplotype, which was not present in individuals without DGI. Haplotype analysis sublocalized the gene to a 5 cM genetic interval that contained the collagen 1A1 gene locus. More than 100 different COL1A1 gene mutations have been associated with various forms of OI, and five of these have been associated with DGI. After excluding these five mutations, we performed mutational analysis of the 51 exons including intron-exon boundaries, which resulted in identification of a Gly559Cys mutation in exon 32, present in all DGI affected family members. Clinical features segregating with this Gly559Cys mutation included hyperextensible joints, joint pain and an increased propensity for bone fractures with moderate trauma. This is the first report of joint pain associated with a COL1A1 mutation and DGI. The mild skeletal features and reduced penetrance of the non-dental findings illustrate the importance of genetic evaluations for families with a history of DGI.

Lack of deletions in the critical region for 11 individuals with atypical Williams-Beuren syndrome. A. Mandel¹, B. Pober², S.W. Scherer³, L.C. Tsui³, L.R. Osborne¹. 1) Medicine, University of Toronto, Toronto, ON, Canada; 2) Yale University School of Medicine, New Haven, CT; 3) Genetics, Hospital for Sick Children, Toronto, ON, Canada.

Williams-Beuren syndrome (WBS) is a multisystem disorder caused by a deletion encompassing 17 genes on chromosome 7q11.23. ELN has been shown to contribute to the cardiovascular defect in WBS but the genes responsible for the remaining features are still undetermined. A number of individuals were identified in our clinics who showed partial WBS phenotypes and we performed analysis of the WBS deletion region to determine whether any had smaller deletions of the region that disrupted critical genes. Eleven patients, ranging from 3 to 18 years of age, were found to have varied subsets of WBS symptoms, with the most common features being developmental delay, hypersensitivity to sound and distinctive facies. When cells from these individuals were studied by FISH with the Oncor probe, all had two copies of ELN present. Ten polymorphic markers from the WBS region were amplified and scored for the presence of one or two alleles. FISH was performed on metaphase chromosomes using genomic clones from throughout the WBS deletion and Southern blot hybridization was performed to search for small deletions. The probes used for Southern blot analysis were CYLN2, GTF2IRD1 and GTF2I, since these were considered most likely to contribute to the WBS phenotype based on previous deletion studies. No individuals had a deletion of the WBS region that was detectable by FISH or polymorphic marker analysis. Southern blot hybridization did not identify any aberrant restriction digestion products that would indicate a junction fragment formed by a rearrangement, nor did they detect any differences in dosage that would indicate a larger deletion. These results suggest that few individuals with non-classical WBS phenotypes have deletions involving genes within the WBS critical region. Their symptoms could be due to small deletions that we failed to detect or point mutations in single genes from the WBS region, however, they likely represent partial phenocopies as a result of the disruption of genes elsewhere in the genome.

DGGE analysis of the tissue non-specific alkaline phosphatase gene in 60 patients/carriers with

hypophosphatasia. *S. Mumm*¹, *J. Jones*¹, *P. Finnegan*¹, *P.S. Henthorn*², *M.C. Eddy*³, *M.N. Podgornik*³, *M.P. Whyte*^{1,3}.

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Hypophosphatasia is a heritable form of rickets/osteomalacia for which there is no established medical treatment. This inborn error of metabolism manifests a wide range of clinical severity spanning death in utero to premature loss of teeth. To delineate the molecular pathology causing the extreme clinical variability and patterns of inheritance of hypophosphatasia, we initiated comprehensive mutational analysis of the gene encoding the tissue non-specific isoenzyme of alkaline phosphatase (TNSALP). Genomic DNA from more than 120 probands, spanning the entire clinical spectrum, is being studied. Using a subset of 10 patients and single-strand conformational polymorphism (SSCP), we detected approximately 70% of the potential mutations (assuming two mutations for each case of autosomal recessive inheritance and one mutation for rare cases of dominant inheritance). This success rate resembles published efficiencies for SSCP. To increase the precision of mutation detection, we have developed the technique of denaturing gradient gel electrophoresis (DGGE) for the TNSALP gene. DGGE primers and conditions were established for all the coding exons (2-12) and adjacent splice sites; the amplified products incorporate a GC clamp on one end. For the subset of 10 patients, we detected 100% of expected mutations using DGGE. Hence, our initial results demonstrate that DGGE analysis significantly outperforms SSCP. To date, at least 65 different mutations (in about 70 patients) have been identified in hypophosphatasia. For our group of 60 individuals studied thus far, including patients and carriers, we have identified approximately 20 previously unreported mutations, further demonstrating the remarkable genotypic variability of the disease. Characterization of the TNSALP mutations in our large patient population will elucidate the molecular pathology and inheritance patterns, and could improve prognostication for hypophosphatasia.

Mutations in the Glaucoma Gene, MYOC, Result in Normal Synthesis but Abnormal Secretion of the Myocilin Protein. *V.C. Sheffield^{1,2}, M. Andrews², A. Shepard³, N. Jacobson³, C. Searby², D.Y. Nishimura¹, Y.H.Kwon Kwon¹, B. Davidson¹, G.S. Hageman¹, W.L.M. Alward¹, A.F. Clark³, E.M. Stone¹.* 1) University of Iowa, Iowa City, IA; 2) Howard Hughes Medical Institute, Iowa City, IA; 3) Alcon Research Ltd, Fort Worth, TX.

The glaucomas are a group of diseases that result in progressive degeneration of the optic nerve, often in association with elevated intra-ocular pressure. The most common form of glaucoma, primary open angle glaucoma, is a leading cause of blindness in which a substantial number of affected individuals have a genetic component. We have previously shown that a glaucoma locus (GLC1A) on chromosome 1q harbors a gene that encodes a 57kd protein known as myocilin. The normal role of this protein and the mechanism by which mutations in the gene (MYOC) cause glaucoma are not known. MYOC mutations have been shown to account for most cases of juvenile onset primary open angle glaucoma, and a small but significant fraction of the more common adult onset disease, in a variety of populations. In order to determine the mechanism by which MYOC mutations cause glaucoma, a number of mutant cDNA constructs were made (including the most common known disease associated mutations) and transfected into several human cell lines using adenovirus vectors and pcDNA3 expression vectors. Proteins were analyzed by western blot analysis with antibodies directed against the native protein as well as against a FLAG tag. Myocilin protein was examined from cell culture supernatants and whole cell lysates. The results show that wild type myocilin was detected in supernatants as well as in whole cell lysates, consistent with the observation that myocilin is a secreted protein. Mutant myocilin was present abundantly in the cell lysates, but was not detected in the supernatants, indicating that the mutant proteins are not properly secreted. A similar defect in myocilin secretion occurs in vivo. Mutant myocilin was absent in the aqueous humor of patients with GLC1A glaucoma, whereas wild type myocilin was expressed abundantly in the aqueous humor of non-glaucoma patients. These studies provide new insight for elucidating the pathophysiology of glaucoma.

Comparative sequencing of aryl-hydrocarbon interacting protein like-1 (AIPL1), a protein associated with Leber congenital amaurosis. *M.M. Sohocki, D.T. Tirpak, S.P. Daiger.* Human Genetics Ctr, Univ Texas-Houston Health Sci Ctr.

Leber congenital amaurosis (LCA) accounts for at least 5% of all inherited retinal disease, and is the most severe inherited retinopathy, with the earliest age of onset. We recently described a novel photoreceptor/pineal-expressed gene, AIPL1, which maps to 17p13 and whose function is unknown. Mutations of AIPL1 cause up to 10% of LCA worldwide, and may cause dominant cone-rod dystrophy. A first step toward identification of a therapy for AIPL1-associated retinopathy is to determine the protein's normal function; comparative sequencing is one approach to detect highly conserved protein subsequences likely to be involved in protein function. Sequencing of human, mouse, rat and cow *aip11* indicated a high amount of protein sequence conservation between species (86% identity, 95% similarity across all species tested) outside of a proline-rich "hinge" region at the carboxyl terminus of primate *aip11*. The protein sequence in all species contains three highly conserved tetratricopeptide (TPR) motifs, 34 amino acid motifs consistent with nuclear transport or chaperone activity. Therefore, a similar function of AIPL1 in the retina is likely. Further, although the hinge region is absent in the mouse, rat and bovine proteins, it is present in all primates tested (human, chimpanzee, baboon, rhesus monkey, squirrel monkey). These proline-rich regions are a common binding motif at the amino or carboxyl termini of proteins, and are thought to bring proteins together in such a way that subsequent interactions are more probable. It is possible that this region of AIPL1 is performing a similar function in primate vision. These data suggest that AIPL1 is performing a role in protein trafficking or folding in photoreceptors and pinealocytes. In addition, these data will be used for several applications, including 1) determination of the likelihood that sequence variants identified in patients cause disease, 2) identification of appropriate animal models of AIPL1-associated retinopathy, and 3) analysis of genotype/phenotype relationships identified for specific AIPL1 mutations. Supported by grants from the Foundation Fighting Blindness and the Hermann Eye Fund.

Late onset Stargardt disease is associated with missense mutations in nonconserved regions of *ABCR*. A.N

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Stargardt disease (STGD, MIM# 248200) is an autosomal recessive macular dystrophy, observed in children or young adults usually before age 30. It is characterized by central visual impairment, progressive bilateral atrophy of the retinal pigment epithelium with frequent yellow flecks surrounding the macula and the midretinal periphery. The estimated frequency of STGD is 1:8000-10000 in the USA. The gene responsible for STGD encodes a photoreceptor-specific ATP-binding cassette transporter (*ABCR*) with 50 exons. Previous surveys reported a spectrum of *ABCR* mutations with different pairings of missense and truncated alleles in early onset STGD patients. To evaluate the influence of the type and position of *ABCR* mutations on late onset disease, we sequenced directly all 50 *ABCR* exons in 28 patients from 25 families with late onset STGD (mean+2SD or >35 years) and completed segregation analysis for identified mutations. *ABCR* mutations were found in 39/56 (70%) disease chromosomes; 28 of 39 (74%) alterations represent missense amino acid changes and include 15 different types of mutations. We report 8 new mutations that were not found in 140 control chromosomes. We identified mutations in both disease chromosomes in 14 STGD patients; in 11 of these subjects we found a combination of missense and truncating alleles, in 3 patients with 36, 40 and 43 years we identified two missense changes. The most common mutations, A1038V and R1108C, were found in 4/39 (10.2%) and 5/39 (12.8%) of mutant alleles. Interestingly, all 28 missense mutations in late onset STGD subjects were found outside of the predicted conserved regions of *ABCR* (transmembrane, ATP-binding or hydrophobic domains), whereas mutations in subjects with early onset STGD have occurred with equal frequency in both conserved and nonconserved regions. These data suggest that late onset STGD is associated with missense mutations in nonconserved regions of *ABCR* that may represent milder mutant alleles. Finally, our data strongly support the hypothesis that the type and the location of mutations in *ABCR*, and the selected combination of mutant alleles, are important determinants of age-of-onset of STGD.

The functional consequences of FOXC1 missense mutations found in Axenfeld-Rieger malformation patients.

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FOXC1 (formerly called FKHL7) is a member of the forkhead family of transcription factors. Mutations of FOXC1 have been found in patients with Axenfeld-Rieger (AR) malformations. We are studying the effect of five different missense mutations of FOXC1 (S82T, I87M, F112S, I126M, and S131L) on the ability of the FOXC1 protein to localize to the nucleus and to bind to DNA. Immunofluorescence and analysis of the localization of FOXC1-Green fluorescent protein hybrid molecules revealed that the wildtype FOXC1 protein is localized to the nucleus when expressed in mammalian COS-7 cells. When the forkhead domain is deleted, FOXC1 protein is localized to the cytoplasm. This is consistent with the hypothesis that the forkhead domain contains nuclear localization signals (NLSs) and with FOXC1's role as a transcription factor. Site-directed mutagenesis was used to introduce into the FOXC1 cDNA all five missense mutations found in AR patients. Analyses of all five mutant FOXC1 proteins revealed that all mutant proteins correctly localized to the cell nucleus. Electrophoretic mobility shift assays have indicated that two missense mutation results in decreased ability to bind to DNA as compared to wildtype FOXC1. One missense mutation produces an unstable protein. One missense mutation results in a FOXC1 protein with DNA binding indistinguishable from that of wildtype FOXC1, and thus might be a rare-non-disease causing mutation. Most interestingly, the last missense mutation results in a FOXC1 mutant protein with 2-3 fold increased DNA-binding ability versus wildtype. These data are consistent with the hypothesis that not only reduced, but increased FOXC1 DNA-binding ability can cause AR malformations. In addition to determining if these missense mutations of FOXC1 are disease-causing, these experiments will help elucidate the manner in which FOXC1 regulates gene function.

Achondroplasia phenotype due to novel FGFR3 mutation. *B.U. Zabel¹, K. Hilbert¹, M. Beck¹, C. Stelzer¹, G. Wildhardt¹, A. Winterpacht², J. Spranger¹.* 1) Dept Pediatrics, Univ Mainz, Mainz, Germany; 2) Department of Human Genetics, University of Hamburg, D-22529 Hamburg, Germany.

The spectrum of disorders resulting from FGFR3 mutations encompasses the achondroplasia skeletal dysplasia family as well as craniosynostosis conditions. The genetic defect accounting for achondroplasia (ACH) is a single amino acid change in the FGFR3 transmembrane domain (G380R in more than 99% of cases). Genetic testing proved one out of 132 typical ACH-patients to be negative for the published ACH (and HCH) mutations. This child is characterized by rhizomelic dwarfism, large head with midface hypoplasia, thoracolumbar kyphosis and lumbar lordosis, short trident hands, mild hypotonia. Radiologic features include short cuboid vertebral bodies with narrowing of lumbar interpedicular distance, squared iliac wings with short sciatic notch, short thick tubular bones with metaphyseal flare and ball-in-socket epiphyseal-metaphyseal junctions. Analysis of the complete FGFR3 coding sequence revealed a missense mutation in exon 7 consisting of an A to G transition at the second nucleotide of codon 278 changing a tyrosine into a cysteine (Y278C). This newly created cyteine residue is located in the extracellular Ig domain IIIa and constitutes the first observation of an ACH mutation outside the transmembrane region. Gain and loss of cysteine residues have been reported in the IgIII loop of FGFR2 resulting in craniosynostotic syndromes. Futher structural and functional studies should help to elucidate the ACH-causing mechanism of the described mutation.

Familial partial lipodystrophy (FPLD), Emery-Dreifuss muscular dystrophy (EDMD-AD) and dilated cardiomyopathy and conduction-system disease (D-CM); lamin A/C mutations and clinical overlap. R.A.

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FPLD (Dunnigan variety) is an autosomal dominant disorder resulting in loss of subcutaneous fat at puberty from the extremities and trunk, and by excess fat desposition in the head and neck. It is frequently associated with profound insulin resistance, dyslipidemia and diabetes. We localized FPLD to chromosome 1q21-q23 with linkage analysis and defects in the lamin A/C gene have been demonstrated to be responsible for the disease. This gene is also altered in the autosomal dominant form of EDMD-AD and in D-CM. The lamin A/C heterodimer comprises a hydrophobic head and tail flanking a rod domain and is associated with the inner nuclear membrane and chromatin. We have detected lamin A/C mutations in all of our FPLD families linked to 1q21. All alterations are mis-sense and most fall within the hydrophobic tail of the A/C heterodimer (6 R482Q; 7 R482W; 1 G465D and 1 K486T). One atypical family harbors an R582H alteration within the C-terminal tail of lamin A only. In this family affected members had less severe loss of subcutaneous fat from the extremities and trunk than usual, and had lower serum triglyceride and higher high-density lipoprotein cholesterol concentrations. EDMD-AD mutations have been found within the rod and hydrophobic tail. D-CM mutations have been found within the rod and within the C-terminal region of lamin C. However, we have observed some degree of overlap in our FPLD families with these other diseases. For example, some patients with alterations of R482 complained of muscle weakness, were diagnosed with muscular dystrophy or had cardiac defects. One FPLD family harbors a mis-sense change within the hydrophobic head of lamin A/C (R28W) and the proband has required a pacemaker at 45 years due to cardiac defects and has complained of muscular weakness. Mis-sense mutations within Lamin A/C may affect Emerin localization and nuclear envelop re-assembly and result in apoptosis of adipocytes and/or myocytes rather than in cell division and differentiation.

Phenotype/Genotype relationships in Usher Syndrome: prevalence of the Usherin 2299delG mutation in patients with an Usher I, II, III or Usher-other diagnosis. *M.D. Weston, W.J. Kimberling.* Genetics, Boys Town National Res Hosp, Omaha, NE.

Usher Syndrome is a genetically heterogeneous disease of retinitis pigmentosa (RP) and hearing loss (HL). Mutations in the extracellular matrix protein Usherin underlie the Usher type IIa genetic subtype (Eudy et al Science 280:1753, 1998). The most common Usherin mutation, 2299delG, has been shown to be a common cause of Usher type II (Weston et al. Am J Hum Genet 66:1199, 2000) and has been observed in patients with atypical Usher syndrome (Lui et al. Am J Hum Genet 64:1221, 1999). Recently, 2299delG mutations have been observed in RP patients with or without mild hearing impairment (Rivolta et al. Am J Hum Genet 66:1975, 2000). To better understand the contribution of Usherin mutations in the spectrum of Usher phenotypes, DNA from patients with a referring diagnosis of Usher I (RP, profound HL, vestibular areflexia), Usher II (RP, moderate-severe HL), Usher III (RP, progressive HL) or Usher-other (RP, HL with other complicating clinical presentations) were subjected to an ARMSs assay developed to test for the presence of the 2299delG mutation. To date, 630 independent patients have been screened; 112 carry 2299delG, 19 being homozygous. The majority of 2299delG positives (18 homozygotes, 86 heterozygotes) were from the Usher II diagnosis group. Five heterozygous Usher III patients, 2 heterozygous Usher-other patients and 1 Usher I homozygous patient were also found. 2299delG frequencies in these populations are as follows: 1/145 USH1, 104/437 USH2, 5/33 USH3, and 2/15 USH4. The data presented confirm the observation that mutations in Usherin cause a clinical presentation of hearing loss that is variable among Usher Syndrome patients. The data also implicates Usherin mutations in perhaps half of Usher III and Usher-other cases where hearing loss is not profound. The prevalence of Usherin 2299delG not only in diverse Usher phenotypes but also in recessive RP argues for general Usherin mutation screening in all patients with RP, regardless of hearing status. This work is supported by grant NIH-NIDCD 2PO1DC01813-07 (W.J.K.) and The Foundation Fighting Blindness (W.J.K.).

Mutations in the fibrillin gene associated with diverse phenotypes in marfan and marfanoid syndromes. *F.V. Schaefer, L. Whetsell.* Dept Molecular Genetics, HA Chapman Institute Medical Genetics, Tulsa, OK.

Marfan syndrome is a connective tissue disorder that affects multiple organs of the body. The latest of several meetings to establish acceptable clinical criterion that would reduce the number of individuals inappropriately diagnosed with Marfan syndrome took place in Ghent in 1996. However, little was known about the mutations in the fibrillin gene that cause Marfan disease, and how they may confirm or influence the clinical definitions. This is especially important with respect to the life-threatening aspects of Marfan syndrome, heart problems. Our study was directed to test the hypothesis that many of the patients in which we found mutations in the fibrillin 1 gene on chromosome 15 did not have sufficient phenotypic traits to meet the Ghent criterion for Marfan syndrome. Patients with one or more clinical signs of Marfan syndrome were collected and fibroblasts were cultured from a punch biopsy. Fibrillin mRNA was isolated, converted to cDNA and the entire fibrillin 1 gene was sequenced. This approach detects splice mutations more efficiently than direct DNA sequencing, but may miss mutations that cause a more rapid turnover of fibrillin mRNA. To date, we have finished testing 45 families and identified a mutation/alteration in 11 of the cases. Almost all of these families had some degree of heart problems in their clinical description. (It should be noted that patient recruitment probably also contributed a degree of bias of ascertainment). However, most also did not meet the standard criterion for Marfan disease as defined by the Ghent conference. In conclusion, a high proportion of patients that do not meet the Ghent criterion for Marfan syndrome have putative disease-causing mutations. This suggests that the clinical criterion for diagnosis may need further examination. The proper diagnosis is especially important since it is the life-threatening heart problems that are a common demoninator in these families. Therefore, DNA testing should be considered in patients that fit some, but not all of the diagnostic criterion for Marfan syndrome. Finally, these families can contribute to further understanding of phenotype/geneotype correlations.

Autosomal recessive Robinow syndrome is caused by homozygous mutations in *ROR2*. A.R. Afzal¹, A. Rajab², C.D. Fenske¹, M. Oldridge³, N. Elanko³, E. Ternes-Pereira⁴, B. Tuysuz⁵, V.A. Murday¹, M.A. Patton¹, A.O.M. Wilkie³, S. Jeffery¹. 1) Medical Genetics Unit, St George's Hospital Medical School, London, UK; 2) Medical Genetics Unit, DGHA, Ministry of Health, Sultanate of Oman; 3) Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK; 4) Departamento de Clinica Medica, Universidade Federal de Santa Catarina, Florianopolis, SC, Brazil; 5) Department of Paediatrics, Cerrahpasa Medical Faculty, University of Istanbul, Turkey.

The Autosomal Recessive form of Robinow syndrome (RRS) is a severe skeletal dysplasia with generalized limb bone shortening, segmental defects of the spine, brachydactyly and a dysmorphic facial appearance. Previously we mapped the gene for RRS to chromosome 9q22, a region that overlaps the locus for autosomal dominant brachydactyly type B. The recent identification of *ROR2*, encoding an orphan receptor tyrosine kinase, as the gene mutated in brachydactyly type B, and the mesomelic dwarfing in mice homozygous for a *lacZ* and/or a *neoinsertion* into *Ror2*, made the gene a suitable candidate for RRS. We found homozygous missense mutations in both intracellular and extracellular domains of *ROR2* in affected individuals from three unrelated consanguineous families, and a nonsense mutation that removes the tyrosine kinase domain and all subsequent 3' regions of the gene in 14 patients from seven families from Oman. The nature of these mutations suggests that RRS is caused by loss of *ROR2* activity.

Mutations in the neurofilament light gene are responsible for axonal form of Charcot-Marie-Tooth disease. *O.V. Evgrafov^{1,2}, I.V. Mersiyanova¹, P. De Jonghe², E. De Vriendt², E.L. Dadali¹, A.V. Perepelov³, V.F. Sitnikov⁴, A.V. Polyakov¹, V. Timmerman²*. 1) Research Center for Medical Genetics, Moscow, Russia; 2) Flanders Interuniversity Institute for Biotechnology (VIB), University of Antwerpen (UIA), Antwerpen, Belgium; 3) State University of Mordovia, Saransk, Russia; 4) Russian State Medical University, Moscow, Russia.

Charcot-Marie-Tooth disease (CMT) is the most frequent hereditary neuropathy with an overall prevalence of 10/100.000. The disorder can be divided into two large groups, CMT1 (myelinopathies) and CMT2 (axonopathies) mainly on the basis of nerve conduction velocity (NCV) criteria. Dominant CMT1 is caused by mutations in myelin protein genes MPZ, PMP22 and connexin 32, or in the EGR2 transcription factor. Mutations in these genes affect myelin structure and electrophysiology. Some patients with a CMT2-like diagnosis may have mutations in MPZ and Cx32. The molecular basis for typical CMT2 is not yet known, since no genes were identified yet. We recently localized the gene responsible for CMT2 in large Russian family on chromosome 8p21 (designated as CMT2E) and found a Gln333Pro (c.998A>C) missense mutation in the neurofilament light gene (NF-L) segregating with the disease. Subsequent screening for mutations (via DHPLC and direct DNA sequencing) in other CMT2 families and isolated patients from different European countries was performed. An additional Pro8Arg (c.22C>A+23C>G) double missense mutation in NF-L gene was found in a large Belgian CMT2 family. This mutation segregates with the disease (maximal lod score is 3.61). Both mutations occur at amino acids, which are well conserved in all sequenced NF-L genes, i.e. from human to frog. These mutations were not found in healthy controls. Neurofilaments are important structural elements of axons and participate in axon transport. They are involved in pathogenesis of several neurological diseases, including amyotrophic lateral sclerosis and Alzheimer disease. However, the pathological mechanism of CMT2E is not clear yet, and identification of additional genes for CMT2 could be helpful for understanding the pathogenesis of CMT.

Three generation family with hereditary hyperferritinemia-cataract syndrome carrying a missense mutation in the L-ferritin gene. *P.M. Czarnecki¹, D.G. Brooks², R. Rao³, J.W. Taub³, D.E. Stambolian⁴, E.V. Bawle⁵.* 1) Greenwood Genetic Center, Greenwood, SC; 2) Division of Medical Genetics, University of Pennsylvania; 3) Children's Hospital of Michigan, Detroit, MI; 4) Department of Ophthalmology, University of Pennsylvania; 5) Division of Genetics, Children's Hospital of Michigan, Detroit, MI.

Hereditary hyperferritinemia-cataract syndrome (HHCS) is a recently identified disease characterized by early onset, bilateral cataracts and elevated serum ferritin levels without iron overload. HHCS is an autosomal dominant condition determined to be caused, in the majority of patients, by mutations within the iron-responsive element (IRE) of the L-ferritin gene. Our patient is a 15-year-old male who was diagnosed at birth with cataracts. He had numerous small punctate opacities including the nucleus of each lens. He has an extensive family history of cataracts including his brother, sister, mother, 3 maternal aunts and a maternal grandmother. He presented at the age of 13 years with bilateral knee and ankle pain. A serum ferritin level was elevated at 1364ng/ml (normal 9.5 to 370 ng/ml), serum iron and serum transferrin saturation were normal. His mother and sister also have elevated serum ferritin levels of 776ng/mL and 570ng/ml respectively (normal range 6.9 - 282.5 ng/mL). A brother without cataracts had normal ferritin levels. Mutational analysis was performed on 3 affected family members and all were found to have a heterozygous DNA sequence change C33T in the L-ferritin IRE. This change has been identified in two other unrelated HHCS families and is known to cause a severe disruption of IRE function by RNA gel shift assay. This case represents the 4th family identified in the United States with HHCS and the first case of HHCS with cataracts documented at birth. The case further indicates the importance of the measurement of serum ferritin levels in patients who have early onset bilateral cataracts characterized by punctate opacities. Patients with hyperferritinemia may benefit from ophthalmologic examination so that they are not confused with other genetic diseases such as hemochromatosis.

Apolipoprotein AII: A novel form of hereditary amyloidosis. *M.D. Benson, J.J. Liepnieks, M. Yazaki, T. Yamashita, K. Hamidi Asl, B. Kluve-Beckerman.* Dept Path & Lab Medicine, Indiana Univ, Indianapolis, IN.

In 1973 Weiss and Page reported a family with amyloid nephropathy characterized by renal glomerular and vascular amyloid. A unique pathologic finding was the presence of glomerular giant cells which were postulated to be involved in resorption of amyloid. The amyloidosis was autosomal dominant with symptoms of renal failure starting in the third or fourth decade. Since that time kindreds have been described with amyloidosis associated with a number of mutant proteins including transthyretin, apolipoprotein AI, fibrinogen Aa-chain, and lysozyme. Despite extensive DNA analysis, affected members of the kindred described by Weiss and Page have not shown any of the previously identified genetic mutations. Recently, a member of the kindred died and tissues were made available for biochemical analysis. Very small amounts of amyloid remained in renal blood vessel walls indicating that glomerular amyloid previously identified on renal biopsy had been resorbed. Amyloid fibrils were isolated, solubilized in 6 M guanidine and fractionated on Sepharose CL6B. Amino acid sequence analysis identified fragments of apolipoprotein AII (apoAII). Western blot analysis of plasma from the patient and his brother identified protein bands reacting with anti-human apoAII with molecular weight of 8,000 and 11,000. Direct nucleotide sequencing of apoAII Exon 4 of the patient revealed heterozygosity for a mutation in the stop codon for apoAII with GGA replacing TGA. This was consistent with a stop to glycine mutation and translation of an additional 22 amino acid residues coded by the 3' untranslated region. RFLP analysis of apoAII Exon 4 PCR products with BstNI identified the new restriction endonuclease site created by the T to G mutation.

Amyloid from apoAII has not been previously identified in humans. This newly discovered human apoAII renal amyloidosis will add to our knowledge of amyloid fibrillogenesis and most likely be of value in evaluating a number of kindreds worldwide with as yet unexplained autosomal dominant amyloidosis.

SCA13, a novel autosomal dominant cerebellar ataxia caused by the expanded polyglutamine in TATA-binding protein identified with 1C2 antibody immunoscreening. *K. Nakamura¹, S.-Y. Jeong^{1, 2}, Y. Ichikawa¹, K.*

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The genetic etiologies of at least 20 percent of autosomal dominant spinocerebellar ataxias (SCAs) have yet to be elucidated. We applied the 1C2 antibody immunoscreening to identify an expanded polyglutamine tract in the lymphoblastoid cell lines from 22 probands that we had already been excluded seven known CAG repeat diseases (HD, SBMA, DRPLA, SCA1-3, 6) by PCR analysis. From antibody screening, we identified a novel polyglutamine protein of ~49 kDa in one pedigree, of which band also reacted to an anti-TBP antibody. PCR analysis of TBP gene revealed abnormal expanded alleles in all affected family members. We further identified seven Japanese families. The phenotype is variable, but the prototypical phenotype begins with a classic spinocerebellar ataxia, and later additional features of dementia and extrapyramidal signs such as parkinsonism and dystonia. This disease resembles the spinocerebellar ataxias or Dentato-rubral pallidolusian atrophy (DRPLA) more closely than any other form of neurodegenerative disorder. Numbers of CAG repeats in TBP gene in affected individuals ranges from 44 to 55, whereas the numbers in normal population (116 chromosomes) ranges from 27 to 42, as previously reported. Age of onset ranges from 19 to 49 years. Most individuals present in the third decade with gait ataxia and dementia. MRI of representative cases indicate diffuse cortical and cerebellar atrophy. By the immunohistochemical study of an autopsied brain sample, we found neuronal intranuclear inclusions (NIIs) with 1C2, ubiquitin and anti-TBP staining, which are thought to be the hallmark of polyglutamine diseases. We designate this novel form of autosomal dominant SCA as SCA13.

Primary LAMP-2 deficiency causes X-linked vacuolar cardiomyopathy and myopathy (Danon disease). I.

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Lysosomal glycogen storage disease with normal acid maltase, originally described by Danon and colleagues, is characterized clinically by cardiomyopathy, myopathy, and variable mental retardation. The pathological hallmark of the disease is intracytoplasmic vacuoles containing autophagic material and glycogen in skeletal and cardiac muscles. Sarcolemmal proteins and basal lamina are associated with the vacuolar membranes. The inheritance of this disease has been considered X-linked dominant because in the majority of the familial cases: 1) males are affected predominantly; 2) in affected mothers, cardiac manifestations are usually milder and later in onset; and 3) no male-to-male transmission has been described. We identified LAMP-2, one of the major lysosomal membrane glycoproteins, as a particularly promising candidate for the defective protein in this disease because "lysosomes" and "membranes" are abnormal in this disorder and because LAMP-2 is the only gene encoding a lysosomal membrane protein known to be on the X chromosome. We sequenced the LAMP-2 gene in DNA from 10 unrelated patients, including one of the probands in the original case report and identified mutations in all patients. Immunohistochemical and Western blot analyses showed LAMP-2 deficiency in skeletal and cardiac muscles. This is the first example of human cardiopathy-myopathy due to mutations in a structural rather than an enzymatic lysosomal protein. The disorders previously described as "lysosomal glycogen storage disease with normal acid maltase" are likely to be genetically heterogeneous. Furthermore, the original description of this disease is probably inaccurate because glycogen is not always increased. Now that the genetic cause of Danon's original case has been identified, we propose to redefine Danon disease as primary LAMP-2 deficiency leading to vacuolar cardiomyopathy and myopathy with or without mental retardation.

Spinal Muscular Atrophy in the South African Black population. *R.W. Labrum¹, J. Rodda², A. Krause¹*. 1) Department of Human Genetics, South African Inst Med Res and University of the Witwatersrand, Johannesburg, Gauteng, South Africa; 2) Department of Paediatrics, Chris-Hani Baragwanath Hospital, Johannesburg, Gauteng, South Africa.

SMA is an autosomal recessive disorder that results in symmetrical muscle weakness and wasting due to the degeneration of the anterior horn cells of the spinal cord. The SMN (survival motor neuron) gene is homozygously deleted in 90-95% of SMA patients. Results from our SMA research in South Africa have shown that only 58.7% (37/63) of black patients with a clinical and pathological diagnosis of SMA have homozygous deletions of the SMN gene. This suggests a different molecular basis for SMA in the SA black population. In order to elucidate the molecular basis, a dosage assay enabling the detection of heterozygotes, has been developed. This assay employs a fluorescent, multiplex PCR reaction with analysis on an automated DNA sequencer. The assay compares the peak area of a number of control bands (genes with two copies) to the peak area of the SMN band to obtain a ratio (2:1-heterozygote, 1:1-non-deleted individual). The dosage assay has confirmed heterozygosity in at least 70% (16/23) of non-deletion patients, suggesting that the majority of black 'non-deletion' patients truly have SMA. Studies are now in progress to attempt to identify the other disease causing mutations in the heterozygote patients. In addition, development of the assay which detects deletion heterozygotes, has enabled us to screen the general population to determine the carrier frequencies in the SA black and white populations. Final frequencies are being calculated. Incorporation of the dosage assay into the diagnostic service will increase the percentage of cases in which the clinical diagnosis can be confirmed as well as improve the comprehensiveness of prenatal and preclinical diagnosis and allow carrier detection.

MHS4R3, a novel putative transcription factor, on chromosome 3q13.1 with relation to the Malignant Hyperthermia Susceptibility Region (MHS-4). *M. Kiehnkopf, J. Schickel, B. von der Goenne, T. Deufel.* Institut fuer Klinische Chemie und Laboratoriumsdiagnostik, FSU-Jena, Jena, Germany.

Malignant Hyperthermia Susceptibility (MHS) is a muscle disorder with autosomal dominant inheritance. Genetic heterogeneity of MHS has been demonstrated and by now, six different loci for MHS have been described. The MHS4 locus was mapped to chromosome 3q13.1; recombinations in affected siblings narrow the MHS4 critical region to a small genetic interval of approximately 1 cM between the markers D3S1563 and D3S1616.

In context with the construction of a PAC/BAC-contig using PAC-library 704RPCI-1,3-5 and BAC-library 753RPCI-11 both derived from RZPD, Berlin, a BAC-clone RPCIB753A201129 was identified containing D3S1616 and EST WI-17954 representing an up to now unidentified transcript. Analysis of the coding capacity of the contig revealed that WI-17954 is also located on PAC-clone RPCIP704I01462 overlapping BAC-clone RPCIB753A201129 and thus located in the MHS4 critical region. RACE-experiments and sequencing of cDNA-clones led to the identification of a novel putative gene product designated MHS4R3. It is encoded by a 3980 bp cDNA resulting in an 881 AA gene product. Screening of multiple tissue cDNA panels using gene specific primers revealed that MHS4R3 is expressed in skeletal muscle as well as in several other tissues. Multiple sequence alignments show that MHS4R3 contains a HMG-BOX domain, a coiled-coil region and a nuclear localization signal which is compatible with the hypothesis that MHS4R3 is a transcription factor. Characterization of MHS4R3 has been started by analyzing the exon/intron structure of the gene. Sequencing of MHS4R3 alleles of MHS-patients is currently under way to further elucidate the potential role of MHS4R3 as a candidate gene in MHS4.

***Rpa4* is a candidate gene for premature ovarian failure.** *K.F Keshav*¹, *F.U. Garcia*². 1) Bioscience, Drexel University, Philadelphia, PA; 2) Pathology, MCP Hahnemann University, Philadelphia, PA.

Epidemiological data suggest there is a strong genetic component involved in determining the age of menopause. Whereas the majority of women experience menopause around fifty years of age, 1% undergo premature ovarian failure (POF), defined as cessation of ovarian function before 40 years of age. Early loss of ovarian function has significant psychosocial consequences and major health implications, including a twofold age-specific increase in mortality rate and significantly higher risk for developing osteoporosis and cardiovascular disease. Abnormalities of the X chromosome are known to be present in many patients affected by POF. Cytogenetic studies of X chromosome aberrations have identified the long arm of the X chromosome, from Xq13 to Xq26, as a critical region for normal ovarian function. It is postulated that interruption of any of eight different genes in this region of the X chromosome will result in POF. The identity of all but one of these genes is still unknown. *Rpa4*, a gene that was cloned by the PI is a candidate gene for POF. There are three pieces of evidence that implicate *Rpa4* in POF. Firstly, *Rpa4* maps to Xq21, the region of the X chromosome critical for ovarian function. Secondly, *Rpa4* is highly expressed in fetal and adult ovarian tissues. In adult ovary *Rpa4* is routinely expressed in the oocyte and has been localized to the nucleus in some oocytes. It is also found in the cytoplasm of granulosa cells. Thirdly, *Rpa4* associates with other members of the RPA protein family (*Rpa1* and *Rpa3*) that have been localized to meiotic chromosomes and are known to be essential for DNA replication, DNA repair and homologous recombination. When associated with *Rpa1* and *Rpa3*, *Rpa4* can bind to single-stranded DNA with high affinity. This affinity for single-stranded DNA supports the proposed role of *Rpa4* in DNA metabolism. *Rpa4* is also expressed in the leydig cells of the testis, implicating *Rpa4* as a protein essential for gonadogenesis. Conceivably, dysfunctional *Rpa4* may disrupt follicle maturation at the point of meiotic chromosome pairing, resulting in elevated rates of follicular atresia and concomitant depletion of follicles.

Molecular analysis of myotilin, the gene responsible for LGMD1A. *M.A. Hauser¹, P. Salmikangas², U.M. Torian¹, R. Dancel¹, L.V.B. Anderson³, U. Taivainen², J.M. Stajich¹, P.C. Gaskell¹, J.M. Vance¹, M.A. Pericak-Vance¹, O. Carpen², M.C. Speer¹.* 1) Duke University Medical Center, Durham, NC; 2) University of Helsinki, Helsinki, Finland; 3) Newcastle upon Tyne, UK.

We have previously reported the identification of the genetic lesion in a large North American family of German descent in which autosomal dominant Limb Girdle Muscular Dystrophy (LGMD1A) is segregating. Symptoms include progressive weakness of the hip and shoulder girdles, as well as a distinctive dysarthric pattern of speech. Affected individuals have a missense mutation in the myotilin gene, which encodes a sarcomeric protein that binds α -actinin, and is localized near the Z-line. Patient muscle exhibits severe Z-line streaming very similar to that seen in nemaline myopathy. Current work is aimed at understanding how the T57I missense mutation seen in affected individuals disrupts muscle function. Western blotting and immuno-staining of frozen sections indicate that myotilin protein is expressed at normal levels in patient muscle, suggesting a gain of function or disruption of a binding interaction with another protein. We have used yeast two hybrid analysis to show that the region of myotilin that binds to α -actinin does not include the T57I mutation. Further, full-length myotilin containing the T57I mutation displays normal binding to α -actinin. This analysis is currently being expanded to map binding sites for other cytoskeletal proteins. We show that the dystrophin-associated glycoprotein complex (DGC) is normal in muscle from LGMD1A patients, indicating that the mechanism by which the myotilin defect causes muscle pathology is entirely distinct from that seen in the autosomal recessive limb girdles. Immunostaining of both the β 1 and γ 1 subunits of laminin is disrupted in LGMD1A muscle. We are examining sarcomeric proteins, the integrins, and components of the extracellular matrix in patient samples using immuno-electron microscopy. We are also constructing a transgenic mouse model for limb girdle muscular dystrophy 1A.

Mutations in aPIX, encoding a guanine nucleotide exchange factor for Rho GTPases, in patients with X-linked mental retardation. *K. Kutsche¹, H. Yntema², A. Brandt¹, I. Jantke¹, H.G. Nothwang³, U. Orth¹, M.G. Boavida⁴, D. David⁴, C. Moraine⁵, H.-H. Ropers³, H. van Bokhoven², A. Gal¹.*

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Molecular analysis of an X;21 translocation in a boy with severe mental retardation and sensorineural hearing loss revealed the breakpoint in Xq26 in a gene (aPIX) coding for a guanine nucleotide exchange factor for Rho GTPases (Rho GEF). Rho GEFs play a key role in organization of the cytoskeleton and mediating neurite outgrowth. To evaluate the possibility that mutations in aPIX might cause nonspecific mental retardation, 119 non-related male patients with nonspecific mental retardation were screened for mutations in the coding region of aPIX. An intronic sequence variant (IVS1-11T®C) was identified in a patient belonging to a large family with disease locus (MRX46) assigned previously to Xq25-q26. RT-PCR amplification of exons 1-4 of the aPIX cDNA of 4 affected males yielded in addition to the wildtype fragment a smaller amplicon in which exon 1 was spliced to exon 3. The ratio between wildtype and truncated RNA was found to be approximately 1:3 in the four patients. Small amounts (3-8%) of exon 2-minus products were also identified in healthy individuals suggesting exon 2-skipping is a rare, but normal event. Analysis of the X-inactivation status revealed a nonrandom pattern in all carrier females in this family. Exon 2 skipping results in an in frame deletion of part of the CH domain, which is required for interaction of aPIX with the actin cytoskeleton. Apparently, cells normally contain a high proportion of aPIX with the CH domain and a small portion without this domain. We are assuming that disruption of the normal ratio between the two aPIX isotypes underlies the pathogenic mechanism in the MRX46 family.

Megacystic, Microcolon, Hypoperistalsis (MMIHS) and Pseudo-Obstruction syndromes: Searching for the human genes. *E. Lev-Lehman, D. Bercovich, W. Xu, A.L. Beaudet.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Megacystis, microcolon, hypoperistalsis syndrome (MMIHS) is a rare autosomal recessive disorder characterized by a marked dilatation of the bladder, microcolon and dilated small intestines. In some patients multiple heart malformation or hydronephrosis were also found. Affected patients usually die early in childhood from various complications including sepsis. Based on the phenotype observed when null mutations were introduced in the mice within the genes coding for the $\alpha 3$ or $\beta 2/\beta 4$ subunits of the nicotinic acetylcholine receptor (nAChR), we are currently conducting a search for mutations in the corresponding human genes. The $\alpha 3$ mutant mice survived to birth, had impaired growth and increased mortality before and after weaning. The most striking phenotype was their enlarged bladder, dribbling urination, bladder infection and widely dilated ocular pupils that do not contract in response to light. They also had distended stomach and bowel. Homozygous null mice for either $\beta 2$ or the $\beta 4$ genes resulted in no visible phenotypic change except that bladder strips from $\beta 4$ mutants did not respond to nicotine, but did respond to a muscarinic agonist, similarly to the $\alpha 3$ mutants. This confirmed that the pathogenesis of the disease involves the nicotinic receptors in the ganglions innervating the bladder. Mice homozygous for the double null had a similar phenotype to the $\alpha 3$ null mice, with enlarged bladder, impaired growth and increased perinatal mortality. Lack of contractility of the bladder appears to be the major cause of morbidity and mortality, similar to human MMIHS condition. Mutation analyses were performed on the human $\alpha 3$ gene and multiple polymorphism in both exons and introns were identified. Since no definitive loss of function mutations were identified in the gene, we are searching for mutations in the $\beta 4$ and $\beta 2$ genes. For this purpose the genomic structure of the human $\beta 4$ gene has been determined and a search for mutations is currently performed using DHPLC and direct sequencing of PCR products. Some amino acids substitutions have been identified but it is not clear if these are pathological or represent benign variants.

Retinal pigment epithelium-expressed genes as putative candidates for inherited retinal dystrophies mapping to chromosome 6q. *P.S. Lagali¹, R. Ayyagari⁴, L.E. Kakuk⁴, I.M. MacDonald^{2,3}, P.W. Wong^{1,2,3}.* 1) Departments of Biological Sciences; 2) Ophthalmology, and; 3) Medical Genetics, University of Alberta, Edmonton, Canada; 4) W.K. Kellogg Eye Center, University of Michigan, Ann Arbor, Michigan.

The retinal pigment epithelium (RPE) is an amitotic cellular monolayer that lies between the neural retina and the vascular-rich choroid layer of the eye. It serves a variety of physical, biochemical, and metabolic functions including maintenance of the blood-retina barrier, phagocytosis of photoreceptor cell outer segments, renewal of retinal for the synthesis of rhodopsin in photoreceptor cells, elimination of metabolic by-products of the visual signal transduction pathway, transport of molecules between the retina and choroid, and maintenance of retinal adhesion. Failure in any of its responsibilities leads to significant changes in the surrounding ocular tissues, ultimately causing retinal degeneration. Primary RPE damage and expression of aberrant RPE-derived factors are both known causes of compromised photoreceptor cell function and survival and underlie several independent retinal dystrophies. We are interested in identifying potential candidate genes for human retinal degenerative disease. Here we focus on an analysis of the long arm of human chromosome 6, a genomic region to which we and others have mapped several retinal dystrophy loci. Expressed sequence tags (ESTs) mapping to a genomic interval on chromosome 6q were obtained and screened by dot blot analysis using human RPE-derived cDNA as a probe. A significant proportion of the ESTs analyzed show expression in the RPE, as confirmed by Northern blot analysis. Selected clones were subjected to further analysis to determine global expression profiles, transcript number and size, and interspecies sequence conservation. Our results indicate the presence of a number of RPE-expressed sequences on chromosome 6q, which, if mutated, may play a role in the etiology of any of the at least nine retinal dystrophies that map to the genomic interval assessed. [Supported by AHFMR, the E.A. Baker Foundation of the CNIB, Foundation Fighting Blindness-Canada, and MRC].

A transient hyperekplexia phenotype associated with compound heterozygote mutations in the human b-subunit of the Inhibitory Glycine Receptor (GLRB). *M.I. Rees^{1,2}, T.M. Lewis³, G. Mortier⁴, R.G. Snell², P.R. Schofield³, M.J. Owen¹.* 1) Department of Psychological Medicine, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, UK; 2) Department of Molecular Medicine, University of Auckland Medical School, Auckland, New Zealand; 3) The Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, Sydney, NSW 2010, Australia; 4) Centre of Medical Genetics, University Hospital Gent 0K5, De Pintelaan 185, B-9000 Gent, Belgium.

Hyperekplexia or startle disease is a neurological disorder characterised by neonatal hypertonia and an abnormal startle response to unexpected stimuli. A proportion of sporadic and familial hyperekplexia patients exhibit dominant or recessive missense point mutations in the $\alpha 1$ subunit (GLRA1) of the inhibitory glycine receptor (GlyRa1). GlyRa1 is a heteropentameric, ligand-gated ion channel composed of three ligand-binding GLRA1 subunits plus two structural b-subunits (GLRB) that cluster on the postsynaptic membrane of inhibitory neurons in the brainstem and spinal cord. Only a proportion of patients exhibit GLRA1 mutations, suggesting that this disorder is susceptible to locus heterogeneity. Consequently, we conducted mutational screening of GLRB exons and flanking intronic regions in a cohort of 22 seemingly sporadic patients with hyperekplexia and hyperekplexia-like conditions. We provide evidence of compound heterozygosity for recessive GLRB mutations, inherited from heterozygous and unaffected parents, in a young boy who presented with typical neonatal hyperekplexia. The maternal mutation (IVS5 +5G®A) is likely to cause aberrant GLRB splicing, whilst the paternal missense mutation, G920A (Gly229Asp), is the subject of electrophysiological characterisation. These mutations are not present in 100 unrelated controls and were not found in the remainder of the cohort sample. At 3 years of age the phenotype has transiently developed into a mild neurological condition, where a startle response is exclusively invoked by tactile stimulus of the perioral region.

Contig assembly and candidate gene identification in the MHS4 critical region on chromosome 3q13.1. J.

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Malignant Hyperthermia Susceptibility (MHS4), a dominant inherited, genetically heterogeneous pharmacogenetic muscle disorder, has been mapped to within a small genetic interval (<1 cM) defined by markers D3S1281/D3S1563 and D3S1616 on chromosome 3q13.1. A YAC contig was constructed and the markers flanking the MHS4 region were identified on a single, non-chimeric YAC clone (939_a_3), obtained from the CEPH Mega-YAC library. Using PAC-library 704RPCI-1,3-5 and BAC-library 753RPCI-11, provided by the RZPD, Berlin, contig construction was performed; pooled DNA from this PAC-library was screened by PCR of STS markers located across the region. BACs and PACs were identified and ordered into two clusters spanning at least 700 kb of genomic DNA with only a single gap with unknown length remaining. Current work is focussed on expanding the contig by "PAC/BAC end walking"; the creation of new STSs from the sequences of the PAC and BAC ends. In parallel, the coding capacity of the region was analysed by screening the contig for ESTs. ESTs were mapped into the framework of markers within the contig and were found to represent at least ten unigene cluster. cDNA clones have been isolated and sequencing is currently under way in order to identify the corresponding genes. Subsequent screening will help to identify the genes playing a role in the pathogenesis of the disease.

Molecular cloning of a t(4;12)(q26;p12) translocation breakpoint in a patient with Andersen syndrome. S.

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Andersen syndrome is characterised by the association of periodic paralysis, cardiac dysrhythmias with prolonged QT interval (long QT syndrome), and variable dysmorphic features. Sporadic as well as familial cases have been reported but no gene has been identified so far. Several long QT syndromes (LQT) have been defined on a genetic basis. To date, five long QT genes have been localised ; four of them (LQT1-3, 5) are now identified and encode potassium or sodium channels. The LQT4 gene has been mapped to chromosome 4q25-q27 but the critical region extends over 18 cM and the gene remains unknown. We have studied a patient with typical Andersen syndrome and having a de novo reciprocal translocation t(4;12)(q26;p12). Both parents were unaffected and did not carry any cytogenetic abnormality. FISH analyses with YACs regularly spaced along the LQT4 region proved that the breakpoint on chromosome 4 actually maps within the LQT4 region, suggesting that the putative gene altered by the translocation event could be LQT4 as well. Additional experiments helped define a region < 700 kb where the breakpoint lies, and several YACs crossing the breakpoint have been identified. Work is in progress in order to identify BACs, PACs and/or cosmids encompassing the breakpoint. Molecular cloning of the breakpoint should lead to identification of a candidate gene for both the Andersen syndrome and the LQT4 syndrome. Moreover, we very recently have shown that several YACs bearing the breakpoint do contain an ion channel gene, making it a very good candidate for both diseases.

Chromosome 15q linked autosomal dominant hereditary spastic paraplegia: new mapping information and candidate gene analysis. *S.R. Rainier, S.M. Jones, M. Bui, J.K. Fink.* Dept Neurology, 5110 CCGC, Univ Michigan, Ann Arbor, MI.

Autosomal dominant, uncomplicated hereditary spastic paraplegia (AD-HSP) is a neurodegenerative disorder characterized by progressive spasticity affecting the legs. AD-HSP is a genetically heterogeneous disorder with known loci on chromosome 14q, 15q, 2p, 8q, 19q, 12q and 2q. The only AD-HSP gene that has been cloned is on chromosome 2p and encodes a gene of unknown function named spastin. We reported the linkage of AD-HSP in a large nonconsanguineous North American kindred of Irish descent to the centromeric region of chromosome 15q (Fink et al., *AJHG* 56:188, 1995). In the present study, we have re-examined the 15q AD-HSP locus by linking a series of new polymorphic markers in the original 126 member family analyzed in the original linkage paper. Analysis of recombination events in affected individuals suggested that the locus is located between D15S128 and the centromere, which moves the locus centromeric to the Angelman syndrome region. Current genetic and physical maps estimate the size of this region to be approximately 6 cM. Included in the region is part of the Prader Willi Syndrome (PWS) region containing imprinted genes, *NDN* and *ZNF127* and the centromeric PWS breakpoint. Also contained in this region is a large duplicon which is duplicated twice telomeric to the Angelman syndrome region. These duplicons are believed to be the mediators of deletions and duplications in PWS/Angelman syndrome patients (Christian et al., *HMG* 8:1025, 1999). There are at least 6 genes of unknown function which are expressed in brain located in this region. One of these genes is in the duplicon and may represent a pseudogene. We have begun mutation analysis of these candidate genes in affected members of the chr 15 linked kindred.

SEARCH FOR SUSCEPTIBILITY GENES OF HUMAN PAPILLOMAVIRUS-ASSOCIATED EPIDERMODYSPLASIA VERRUCIFORMIS ON CHROMOSOMAL REGIONS 2p21-p24 AND 17q25. N.

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Epidermodysplasia verruciformis (EV. MIM#226400) is a rare autosomal recessive genodermatosis. EV is characterized by an abnormal predisposition to infection with a specific group of related human papillomavirus (HPV) genotypes, including the oncogenic HPV5 associated with the skin carcinomas observed in about half of the patients. EV is thus a model to understand the still unknown genetic factors involved in the control of infections with HPVs, in particular the widespread oncogenic HPV genotypes associated with invasive cervical carcinoma. We recently mapped a first EV susceptibility locus (EV1) to chromosomal region 17q25 within the 1-centiMorgan (cM) interval between markers D17S939 and D17S802 and a second locus (EV2) to region 2p21-p24 within the 8-cM interval between markers D2S171 and D2S2347. Linkage in families are restricted to EV1 or to EV2 points to a non-allelic heterogeneity of the disease. We are currently studying additional families and refining the genetic interval of EV1 and EV2 loci to identify candidate EV genes. Further analysis of EV families disclosed a linkage with EV1 in four new families and suggest the existence of a third EV locus in two related families. In silico analysis of the 17q25 region allowed us to map ten novel microsatellite markers and to refine the EV1 locus. Analysis of available nucleotide sequence data of this region led to identify putative genes, including the known genes encoding a septin-like protein and the thymidine kinase-1. Previously to search for mutation segregating with the disease, the expression of these putative genes in the skin and in lymphoblastoid cell lines is ongoing study. The identification of genes conditioning susceptibility to oncogenic HPV genotype infection is now within reach.

p57^{KIP2} mutations associated with loss of IGF2 imprinting in Beckwith-Wiedemann syndrome. *R. Weksberg¹, M. Li¹, Y.L. Fei¹, C. Shuman¹, D. Chitayat¹, J. Atkin³, R.M. Pauli⁴, J.A. Squire².* 1) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, University of Toronto, Toronto, ON, Canada; 2) Ontario Cancer Institute, University of Toronto; 3) Genetics & Birth Defects Center, Morristown Memorial Hospital, Morristown, NJ; 4) Clinical Genetics, University of Wisconsin, Madison, WI.

Beckwith-Wiedemann syndrome (BWS) is an imprinting disorder characterized by somatic overgrowth, congenital malformations and predisposition to childhood tumors. Aberrant expression of multiple imprinted genes, including H19, IGF2 and the maternally-expressed cyclin-dependent kinase inhibitor p57^{KIP2} (CDKN1C), has been observed in BWS patients. It has been estimated that mutations in p57^{KIP2} occur in 12-17% of BWS patients. We have screened 6 autosomal dominant pedigrees and 40 sporadic BWS cases by PCR/heteroduplex analysis and DNA sequencing and have identified three mutations with unusual features, two of which were associated with biallelic IGF2 expression and normal H19 imprinting. Significantly, the majority of AD pedigrees did not involve p57^{KIP2} mutations. In one patient, a nonsense mutation within the QT domain was also identified in the patient's father, identifying a case where paternal transmission of a mutation in a maternally-expressed gene can result in a disease phenotype. A second proband carries a frameshift mutation in the QT domain and is the child of one of a pair of monozygotic twin females who carry the mutation *de novo*. The third patient carries a missense mutation resulting in a leucine to proline substitution in the Cdk inhibitory domain and is associated with cardiac abnormalities and skeletal changes - clinical features more commonly found in other overgrowth syndromes. When considered with other studies published to date, this work reveals the frequency of p57^{KIP2} mutations in BWS to be only 6.5%. This is the first report of an association between p57^{KIP2} mutations and loss of IGF2 imprinting. This finding supports the hypothesis that BWS results from an imbalance between imprinted growth promoting and growth suppressing genes and raises the possibility that p57^{KIP2} may affect IGF2 imprinting.

Genetic Heterogeneity in Familial Hypobetalipoproteinemia. *B. Yuan, C. Gu, S.H. Duan, M. Aversa, R. Neuman, G. Schonfeld.* Washington University School of Medicine, St. Louis, MO.

FHBL is characterized by extremely low plasma levels of apolipoprotein B (apoB), and low density lipoprotein (LDL) cholesterol inherited as an autosomal dominant trait. The apoB gene(2p23-24) is the first identified locus for FHBL. We previously reported a linkage to 3p21.1-22 in a FHBL kindred. In an effort to replicate the linkage finding in this region and to refine the mapping resolution, we genotyped and analyzed 142 individuals in 24 small St. Louis families with FHBL (29 affected individuals). LOD score analysis, homogeneity tests, and haplotype analysis all demonstrated that 8 (of the 24) families are linked to the same region as reported before. A combined lod score of 3.75 at recombination fraction ($q=0$) was obtained for D3S3647. Analyses of linkage to the 3p region conditional on the linkage information at the apoB locus suggest that the two loci may contribute independently to the trait in some kindreds. A haplotype was observed segregating with disease in these linked families, placing the candidate gene between markers D3S2407 and D3S1588. These confirmed our previous finding that 3p21.1-22 may harbor a gene responsible for FHBL, and that FHBL is a heterogeneous genetic disorder. Further analysis using quantitative trait and physical mapping are currently in progress.

Candidate Gene Analysis in Hereditary Lymphedema. *M. Kimak*¹, *E. Lawrence*¹, *K. Levinson*¹, *K. Alitalo*², *M. Kärkkäinen*², *R. Ferrell*¹, *D. Finegold*^{1,3}. 1) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Molecular/Cancer Biology Lab., Haartman Institute, Univ. Helsinki, Helsinki, FI; 3) Dept Pediatrics, Univ Pittsburgh, Pittsburgh, PA.

First described by Milroy in 1892, hereditary lymphedema (HL) generally shows an autosomal dominant pattern of inheritance with reduced penetrance, variable expression, and variable age of onset. Recently, three independent laboratories have reported linkage between chromosomal region 5q34-q35, the location of vascular endothelial growth factor-3 receptor (VEGFR-3), and HL. VEGFR-3 is known to be expressed in lymphatic endothelia. We have identified four independent VEGFR-3 mutations in HL families, each resulting in loss of receptor protein kinase activity. This evidence supports the assertion that genes in the vascular endothelial growth factor/receptor pathway are critical to normal lymphatic development and are biologically plausible candidate genes for HL. Additionally, the homeobox gene, Prox1, has been implicated as a specific, and critical regulator of lymphatic development. To test their potential role in hereditary lymphedema, we performed detailed sequence analysis of the VEGFR-3 ligand, VEGF-D, and Prox 1 in a large group of HL families who did not show linkage to chromosomal region 5q34-q35. Direct sequencing of the exonic regions of these genes did not detect any potentially functional mutations. This data suggest that mutations in VEGF-D and Prox 1 are not a common cause of hereditary lymphedema.

Molecular heterogeneity of ceroid lipofuscinoses in France. *C. Caillaud*^{1,2}, *J.P. Puech*¹, *J. Manicom*¹, *A. Kahn*², *L. Poenaru*^{1,2}. 1) Dept Genetique, CHU Cochin, Paris, France; 2) INSERM U129, Paris, France.

Neuronal ceroid lipofuscinoses (NCL) are inherited neurodegenerative disorders characterized by the accumulation of autofluorescent lipopigments in various tissues. Their main clinical signs are psychomotor retardation, impaired vision, seizures and premature death. Four NCL forms are distinguished according to clinical and morphological features : infantile (INCL), late-infantile (LINCL), juvenile (JNCL) and adult (ANCL). Eight different loci have been described as responsible for the various forms of the disease. Infantile NCL is caused by defects in the CLN1 gene, encoding a palmitoyl protein thioesterase. Four genes are involved in LINCL : CLN2, encoding tripeptidyl-peptidase I (TPP-I), in the classical form, CLN5, encoding a transmembrane protein of unknown function, in a Finnish variant, and two not yet cloned genes, CLN6 and CLN7. In JNCL, the product of the defective gene, CLN3, is a lysosomal transmembrane protein, probably involved in the vacuolar pH homeostasis. The gene responsible for the adult form, CLN4, remains to be identified. Recently, progressive epilepsy with mental retardation (EPMR) has been recognized as a new NCL subtype, due to mutations in the CLN8 gene encoding a putative transmembrane protein. Twenty LINCL and four JNCL patients were tested for the tripeptidyl-peptidase I activity. Thirteen LINCL patients and one JNCL exhibited a TPP-I deficiency. These patients were tested for CLN2 mutations, using PCR-amplification and sequencing of the CLN2 gene. Previously described mutations were found : 3556 G-->C splice mutation (30.8% of alleles), Arg208Stop (27%), 4288-4295del (one allele) and Cys365Tyr (two alleles). Various novel mutations were characterized on the other alleles : Arg339Trp, Trp548Stop, Gln509Stop, 4635 A-->G at the 3 splice junction of intron 8 and a 11-bp deletion in exon 13 (6082-6092del). The TPP-I deficient JNCL patient is a compound heterozygote for the common splice mutation 3556 G-->C and the novel point mutation Ser153Pro. Our results demonstrate the involvement of different loci in the pathogenesis of LINCL and the heterogeneity of CLN2 mutations responsible for LINCL or rarely JNCL.

Conversion of the glucocerebrosidase pseudogene by the functional gene: implication for molecular diagnostic assays. *R. Mao, J.F. O'Brien, K. Snow.* Dept Molecular Genetics, Mayo Clinic, Rochester, MN.

Molecular genetic testing for Gaucher disease, deficiency of the lysosomal enzyme glucocerebrosidase (EC 3.2.1.45), is offered by many molecular diagnostic laboratories. Although more than 130 mutations in the gene for human glucocerebrosidase have been described, most laboratories test for only a few common mutations. Assay design is complicated by the presence of a highly homologous pseudogene. Typically, assays use PCR primers that are specific for functional gene sequences followed by mutation detection using restriction enzyme analysis or an ASO format. Previously we reported compound heterozygosity for N370S and a rare 55bp deletion in exon 9 of the glucocerebrosidase gene in a 28 y/o moderately affected Gaucher patient of German ancestry (Mao et al, 1999). To determine the frequency of this mutation, we tested 184 samples that were referred for carrier screening. Initial testing suggested that 7 samples contained the deletion. However, additional sequence analysis revealed that all of these new cases represented false positive results due to probable gene conversion of the pseudogene. Assay artifact was generated because conversion of pseudogene to functional gene sequence led to the functional gene-"specific" primers being able to amplify the pseudogene. Sequencing between pseudogene nucleotides g.3934 and g.4920 (functional gene nucleotides g.6341 to 7383) was performed using both pseudogene-specific and functional gene-specific primers. Results suggest 3 different boundaries for gene conversion among the 7 cases. Although gene conversion from functional gene to pseudogene sequences is well known (for example, as the putative origin of IVS2+1 and L444P mutations), conversion from the pseudogene to the functional glucocerebrosidase gene has not been reported. Our findings have important implications for the accuracy of PCR-based assays for mutations in genes that have pseudogene(s): false positive results could be generated due to "reverse" gene conversion of the pseudogene by the functional gene sequence. We found evidence for "reverse" gene conversion in 4% of samples, suggesting that this may be a relatively frequent event.

INTRACELLULAR TARGETING OF THE CLN5 PROTEIN AND CONSEQUENCES OF DISEASE MUTATIONS RESULTING IN VARIANT LATE INFANTILE NEURONAL CEROID LIPOFUSCINOSIS. J.

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Finnish variant late infantile neuronal ceroid lipofuscinosis (vLINCL) is caused by mutations in the CLN5 gene, which encodes a polypeptide of 46 kDa with two potential transmembrane domains. Four disease mutations have been characterized, of which Tyr392Stop is the Finnish founder mutation and caused by del(AT)2467 (FINM). This mutation leads to a truncated protein lacking 16 amino acids in the C-terminus. Another Finnish mutation, Trp75Stop, is caused by G1517A transition (Finm). The third mutation, G2127A (EUR), in a Dutch patient, causes an amino acid change of Asp279Asn. The fourth mutation, ins(C)1961 (SWE), leads to a premature stop at amino acid position 253, having 29 missense C-terminal amino acids. One Swedish and one Finnish patient are compound heterozygotes for this mutation. Immunofluorescence staining of transiently transfected COS-1 cells revealed co-localization with a Golgi marker suggesting that CLN5 is mainly targeted to Golgi complex. Some ER staining was also systematically observed. Also FIN and EUR polypeptides get targeted to Golgi complex, whereas SWE is mainly retained in the ER. The half-life of WT, FINM and EUR is approximately 2 h, whereas the SWE mutation leads to a more unstable polypeptide with a half-life of 30 min. The glycosidase treatments of radiolabeled proteins suggest that CLN5 is a high-mannose glycosylated polypeptide carrying more than one N-glycosylation site. No evidence for proteolytic processing of the CLN5 polypeptide can be observed. To elucidate the pathological cascades in the NCL-diseases we performed co-immunoprecipitation analyses of all known CLN proteins. These experiments revealed that CLN5 physically interacts with CLN2 and CLN3, but not with CLN1.

Positional candidate gene identification for keratolytic winter erythema (KWE) on 8p22-23. A.S. Bergheim¹, E. Ogilvie¹, S. Arndt¹, H. Napier¹, M. Taylor¹, A. Simmons², M. Lovett², W. Hide³, M. Ramsay¹. 1) Dept. Human Genetics, SAIMR and University of the Witwatersrand, Johannesburg, South Africa; 2) Dept. Human Genetics, Washington University School of Medicine, St. Louis, USA; 3) South African National Bioinformatics Institute, University of the Western Cape, Bellville, South Africa.

Keratolytic winter erythema (KWE) (MIM 148370) is an autosomal dominant disorder of skin keratinization characterised by recurring cycles of erythema, hyperkeratosis, and blistering. It is particularly prevalent on the palms and soles, and appears to be more severe in colder weather. A 6cM region on chromosome 8p22-23 between markers D8S550 and D8S265 was identified by linkage analysis as containing the KWE gene. Haplotype analysis reduced this region to 1cM, between markers D8S550 and D8S265. cDNA direct selection was used to identify positional candidate genes. Fetal brain (Fb) and salivary gland (Sal) cDNA libraries were screened against a genomic YAC contig (770E9, 915H4, 737E5, 773G4) spanning the region from D8S520 to D8S1130. From each library 960 clones were selected, of these 447 Fb clones and 359 Sal clones were sequenced. Of the sequenced clones, 160 (35%) Fb and 136 (37%) Sal were mapped to a BAC contig (337E21, 493P15, 573G21, 271O23, 367I24, 269E3, 327I14, 358B10) spanning a region from AF124273 to AF124295, containing the 1cM critical region. Thus far we have identified six transcriptional units in this region: the B-lymphocyte kinase (*BLK*) gene; a new member of the elongation factor 1 alpha (*EEF1A*,) family; the human homolog of the *Mus musculus* gene *Amac1*; and three unique transcriptional units defined by the GenBank accession numbers AL080178, HSZ99359, and AA488159. The availability of genomic sequence will facilitate further positional candidate gene identification in this region and these will also be evaluated as candidates for the KWE gene.

Identification of a novel mutation in patient with carnitine-acylcarnitine translocase (CACT) deficiency. J-H.

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The carnitine-acylcarnitine translocase (CACT) deficiency, one of the most severe fatty-acid oxidation disorders, is characterized by hypoketotic hypoglycemia, hyperammonemia cardiac abnormalities and early death. In this study, the proband is the baby girl of consanguineous Mexican parents. At 38 hours of life, the baby had bradycardia and was dead at 4 days. In vitro probe analysis with [16-²H₃]palmitic acid from the proband's fibroblasts and from amniocytes of the current pregnancy resulted in labeled acylcamitines consistent with either carnitine-acylcarnitine translocase or carnitine palmitoyltransferase II (CPT II) deficiency. CACT activity was measured and found to be absent in both fibroblasts and amniocytes. In order to investigate the molecular basis of CACT deficiency, RT-PCR was performed to amplify the CACT coding region. In both the proband and her affected sibling, aberrant CACT cDNA species were present, including exon 3 skipping and 13-bp insertion at cDNA position 388. To examine these cell lines for mutation affecting CACT RNA processing, CACT gene sequences, including intron and exon boundaries, were amplified and sequenced directly. One nucleotide G deletion at the donor site in intron 3 was identified, which led to exon skipping and a 13-bp insertion resulting in truncation of the protein. Both proband and her affected sibling were homozygous for this deletion. This deletion was not detected in 20 normal control subjects by PCR/HphI Restriction-endonuclease analysis. We propose that this mutation of the CACT gene may play a causative role in the disease.

Genotypic and clinical characteristics of a cohort of 144 haemochromatosis patients in western Brittany, France.

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Hereditary haemochromatosis (HHC) is the most common autosomal recessive disorder in populations of Caucasian origin, with an incidence of 1 in 300. Characterised by an iron overload, the disease is associated with increased risk of hepatocarcinoma and the treatment is carried out by venesections. A candidate gene for HHC (HFE gene) was cloned in 1996 and three mutations have been described: the C282Y, present in 70 to 95% of patients, the H63D and the S65C. In this study, we described the genotypic and clinical characteristics of 144 HHC patients treated in a blood center of western Brittany, France. The diagnosis was made on the basis of at least two increased iron parameters (transferrin saturation, ferritin, iron) added by a minimal iron extraction of three grams. In this cohort, 79.9% of patients were males (sex-ratio=3.97). The age at onset was significantly higher in females (52.7 y. versus 46.6 y. - $p<0.01$), but did not differ according to the circumstances of HHC detection. The diagnosis was mainly made on clinical features (63.3%), on family testing (25%) or during an occupational medicine visit (10.4%). The most common symptoms associated at the time of onset were: fatigue (61.2%), arthritis (49.6%) predominantly in females, skin pigmentation (39.2%), metabolic disorders (21.6%) and hepatomegaly (18.2%). The patients achieved iron depletion after 39 venesections of 400 ml in mean. In this cohort, 91% of patients were homozygous for the C282Y mutation, 3.5% were compound heterozygous C282Y/H63D and 0.7% homozygous for the H63D. Only 3.5% of chromosomes did not carry any HFE mutation: four patients presented a single mutation (C282Y/-: n=2, H63D/-: n=1, S65C/-: n=1) and three no mutation. However, among five of those seven patients, we observed other factors known to influence iron parameters (excess weight, excessive alcohol consumption). In this retrospective study based on non-invasive criteria, the HHC diagnosis is closely correlated with the presence of mutations in the HFE gene.

RETT Syndrome diagnostic testing by DHPLC and sequence analysis of the MECP2 gene. *I.M Buyse^{1,2}, P. Fang^{1,2}, K. Hoon^{1,2}, R. Amir², H. Zoghbi^{2,3,4}, B.B. Roa^{1,2}.* 1) Baylor DNA Diagnostic Laboratory; 2) Dept. of Molecular & Human Genetics; 3) Dept. of Pediatrics; 4) Howard Hughes Medical Institute, Baylor college of Medicine, Houston, TX.

Rett syndrome is an X-linked dominant neurodevelopmental disorder that affects females and is usually lethal in males. The disease locus was mapped to the MECP2 gene on chromosome Xq28. To date, MECP2 sequence analysis identified mutations in ~80% of diagnosed Rett patients. Different missense, nonsense and frameshift mutations were identified, with the majority in the methyl CpG-binding or transcriptional repression functional domains in both familial and de novo cases. A diagnostic test was developed to analyze the entire MECP2 coding region by sequencing both forward and reverse directions. To date, a total of 189 unrelated cases (185 females, 4 males) with a possible diagnosis of Rett syndrome were tested in our diagnostic laboratory. A MECP2 coding region mutation was identified in 86/185 (46.5%) female patients of this heterogeneous group and in none of the males tested. Unclassified amino acid substitutions were found in 5/185 patients and analysis of both parents was recommended to evaluate these variants as de novo mutations or polymorphisms. A total of 29 different mutations (6 missense, 8 nonsense, 1 splice-site and 14 frameshifts) were identified. Of these, 14 were novel and 7 were recurrent mutations. A total of 9 polymorphisms were detected. Prenatal diagnosis for a subsequent pregnancy was performed in 2 cases wherein a familial mutation was identified. Parental analysis suggested a de novo mutation and prenatal diagnosis was negative in both cases. Evaluation of the Denaturing High-Performance Liquid Chromatography (DHPLC) test sensitivity showed 100% concordance with sequence analysis for a total of 57 sequence variants analyzed. Thus, we devised a strategy whereby we use DHPLC for initial sequence variation screening. DHPLC variants will be sequenced for mutation identification. For samples that are negative by DHPLC or are found to encode a polymorphism, the entire MECP2 coding region will be sequenced. This provides a robust and efficient strategy for Rett diagnostic testing.

A rapid and reliable method for the precise characterization of the *CFTR* intron 8 polyvariant locus. M.J.

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Cystic Fibrosis (CF) is a common autosomal recessive genetic disorder that can affect lung and exocrine pancreatic function resulting in chronic pulmonary disease, with or without pancreatic insufficiency, and male infertility. A mutation in the exon 9 splice branch/acceptor site of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene affects the proportion of transcripts containing exon 9. This complex mutation in *CFTR* intron 8 consists of a poly T tract of 3 known lengths (T5, T7, T9), and an immediately upstream poly TG tract of 5 known lengths; (TG)₉, (TG)₁₀, (TG)₁₁, (TG)₁₂, (TG)₁₃. These variable tracts could occur in any of 15 combinations. The length of the poly T tract is directly related to splicing efficiency of exon 9, and the length of the poly TG tract is inversely related to splicing efficiency of this exon. The (TG)₁₂/T5 and (TG)₁₃/T5 alleles, in particular, have been found at an elevated frequency among males with obstructive azospermia or congenital bilateral absence of the vas deferens (CBAVD), as well as individuals with cystic fibrosis, idiopathic pancreatitis, and bronchiectasis. Most routine diagnostic testing provides the length of the partially penetrant poly T tract in isolation. We have developed a simple single-tube multiplex method for the simultaneous sizing and phasing of poly T and poly TG tracts. This method allows a more accurate prediction of phenotype than assays that are restricted to the length of the poly T tract. It is also far less time-consuming, costly, and error-prone than sequence-based detection of both repeat elements. We have applied this procedure to more than 100 samples from patients referred for cystic fibrosis, pancreatitis, bronchiectasis, and male infertility testing and have found clear correlations between the polyvariant tract lengths and clinical presentation. It is recommended that diagnostic laboratories routinely implement a screening procedure for both components of this polyvariant locus.

Yeast frataxin inhibits iron-induced hydroxyl radical formation. *J. Adamec, C. Lesnick, G. Isaya.* Departments of Pediatric and Adolescent Medicine and Biochemistry & Molecular Biology, Mayo Clinic and Foundation, Rochester, MN.

Frataxin is a nuclear-encoded mitochondrial protein widely conserved among eukaryotes. Human frataxin is severely reduced in Friedreich ataxia (FRDA), an autosomal recessive neurocardiodegenerative disease. The yeast frataxin homologue (Yfh1p) has been shown to play an important role in mitochondrial iron homeostasis and protection from iron-induced free radical formation. Evidence of increased iron deposits and oxidative damage in FRDA suggests that human frataxin may have a similar role. We have tested the ability of frataxin to prevent iron-induced oxidative damage *in vitro*. Iron-induced formation of thiobarbituric-acid-reactive substances (TBARS) from deoxyribose was measured in the presence of recombinant yeast frataxin (mYfh1p). TBARS formation induced by 30-90 mM Fe^{2+} was 65-20% inhibited by mYfh1p concentrations as low as 0.2 mM, with an I_{50} value of 0.38 mM mYfh1p at 60 mM Fe^{2+} . The antioxidant potential of mYfh1p is higher than that of serum albumin (9% protection with 0.4 mM albumin at 60 mM Fe^{2+}) and tannic acid (30% protection with 10 mM tannic acid at 60 mM Fe^{2+}). The possibility of a non-specific effect due to the acidic nature of mYfh1p ($\text{pI} = 4.34$) was excluded by showing that calmodulin ($\text{pI} = 4.09$) has no antioxidant properties (0% protection with 0.4 mM calmodulin at 30 mM Fe^{2+}). At mYfh1p concentrations ranging from 0.2-1.0 mM, the maximum protective effect was obtained at a Fe:mYfh1p molar ratio of 150:1. The protective effect of mYfh1p was inversely proportional to the iron concentration and remained nearly unchanged at increasing deoxyribose concentrations, indicating that mYfh1p acts more like an iron "chelator" than a free radical scavenger. We have shown previously that Fe^{2+} induces self-assembly of mYfh1p into higher order multimers that sequester over 3,000 atoms of iron in a soluble and available form. The present data indicate that iron sequestration by mYfh1p also limits hydroxyl radical formation from Fenton reaction.

Male letality in CMT1A transgenic mice suggests an effect of a male specific modifier gene. *A. De Sandre, E. Passage, V. Sanguedolce, N. Levy, M. Fontes.* Inserm U491, Genetique Medicale et Developpement, Faculte de Medecine Timone, Marseille, France.

In order to localise and identify modifier genes involed in the clinical heterogeneity of Charcot-Marie-Tooth type 1A disease, we initially analysed the phenotypes of the C22 mouse strain carrying seven copies of the PMP22 human transgene (Huxey et al., 1996 and 1998). The YAC transgene was first mapped on the mouse distal chromosome 12. Surprisingly, we observed a high rate of male letality arising before 9 monthes of life. A statistical analysis (KHI2) was performed and evidenced that male letality was significantly higher among transgenic males when compared to transgenic females and non-transgenic controls in the same sibships. This observation raises different hypothesis : a possible explanation should be the interruption of a gene involved in male specific letality at the transgene integration's site; a second hypothesis is the presence within the transgenic YAC containing the PMP22 gene, of a gene differentially expressed between males and females whose the surexpression leads to a male specific letality. Finally, the existence of a modifier gene not beeing related with the transgene integration is also possible and a male specific letality could be related in particular to an X-linked gene. These hypothesis have all been adresssed and our data suggest the presence of a modifier gene involved in male letality. Analysis of two different candidate genes either mapped on the X chromosome or in the transgenic YAC, syntenic to the CMT1A region in human is in process. Our data will have possible implications for understanding the molecular mechanisms involved in CMT1A clinical heterogeneity in human.

Generation of mice that over-express the Syntaxin 1A protein. *L.R. Osborne¹, T.L. Campbell¹, P. Pasceri², J. Ellis², L.C. Tsui³.* 1) Medicine, University of Toronto, Toronto, ON, Canada; 2) Developmental Biology, Hospital for Sick Children, Toronto, ON, Canada; 3) Genetics, Hospital for Sick Children, Toronto, ON, Canada.

Syntaxin 1A is an essential component of the pre-assembled vesicle docking and fusion machinery necessary for exocytosis. STX1A is also hemizygously deleted in Williams-Beuren syndrome (WBS) and it is postulated this might explain some of the neurological features seen in the disorder. In addition, STX1A has been shown to regulate the CFTR protein either by direct interaction or by affecting recycling of CFTR between the apical membrane and cytoplasmic vesicles. In an attempt to learn more about the role of STX1A in WBS or in cystic fibrosis, we generated a mouse model that expresses human STX1A in addition to the endogenous mouse gene. A cosmid clone containing the entire human STX1A gene in its genomic context was used for direct microinjection into 1 day embryos of FVB mice. Four founder mice that had integrated the transgene in different copy numbers were produced, three of which expressed the transgene as evidenced by RT-PCR of a 300 bp portion of the human STX1A 3' UTR from brain total RNA. Western blot experiments, using monoclonal antibodies for human or mouse STX1A, showed a definite but modest (50%) increase in protein expression compared with non-transgenic animals. The transgene expression mimicked the endogenous expression pattern of *Stx1a*, with major mRNA and protein expression in the brain, plus detectable expression in the pancreas and lung. The assay may not be sensitive enough to detect protein in other tissues if it is present at low levels or in specific cell types. Several heterozygous transgenic animals have died prematurely at around 6 weeks of age from unknown cause and preliminary results show some dilatation of mucous glands in the gut. The mice are currently undergoing detailed histopathological, behavioural and electrophysiological analysis. We postulate that over-expression of STX1A may interfere with normal exocytosis and so disrupt the insertion of proteins into the apical membrane of epithelial cells in tissues such as the lung and gut, leading to ion imbalances.

A killifish model to study the role of CFTR in absorptive and secretory epithelia. *J.E. Mickle, G.R. Cutting.*
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Cystic fibrosis (CF) is a disorder of epithelial cell ion transport caused by mutations in the CF transmembrane conductance regulator (CFTR). CFTR is a key component in the absorption and secretion of electrolytes across airway epithelial cells. Absence or dysfunction of CFTR alters the salinity of airway surface fluid that initiates a cascade of events which culminate in life-limiting pulmonary disease. To determine whether CFTR plays different roles in epithelial absorption and secretion we investigated CFTR in *Fundulus heteroclitus* (killifish), a euryhaline estuarine teleost capable of rapid adaptation from fresh water (absorptive) to seawater (secretory) environments via specialized mitochondria-rich cells (chloride cells) in gill and opercular epithelium. To examine if chloride cells are the site of killifish (kf)CFTR expression, tissue was dissected for immunocytochemistry. Since antibodies specific for kfCFTR have not been developed, antibodies directed against homologues that share the highest degree of identity (shark: 60%, human: 59%) were used. Samples were frozen in embedding medium, sectioned and incubated with primary antibodies for CFTR (60.1.2 and 76.1.2) and for several mitochondrial markers (Hsp60, Bcl-2, Cpn10). Signal was detected with both CFTR antibodies in mitochondria-rich chloride cells of gill and opercular epithelium. Signal was not detected in the absence of primary antibody. To investigate the biosynthesis of native CFTR tissue was collected for Western analyses. kfCFTR was detected with an antibody specific for the carboxy terminal amino acids DTRL (clone 24.1, R&D Systems, Minneapolis, MN) by chemiluminescence. Signal was sized against molecular markers run in parallel and antibody specificity was confirmed with human and shark CFTR controls. kfCFTR from gill migrated as two forms approximately 160 kDa and 210 kDa in size. kfCFTR from the operculum was detected as a single band at 160 kDa. The molecular mechanism underlying these isoforms is being investigated. These results indicate that killifish is a suitable model to assess the role of CFTR in tissues that can easily be manipulated to absorb or secrete electrolytes.

Using murine strain variations to study the regulation of CFTR expression. *S.M. Satinover¹, T.R. Romigh², M.L. Drumm^{1,2}*. 1) Genetics, Case Western Reserve University, Cleveland, OH; 2) Pediatrics, Case Western Reserve University, Cleveland, OH.

Cystic Fibrosis (CF) is caused by defects in CFTR, the cystic fibrosis transmembrane conductance regulator, a cAMP-regulated chloride channel. Although there have been great advances in the understanding of the genetic, biochemical, and cellular biology of CFTR, the regulation of gene expression is poorly understood. We have recently identified differential tissue-specific allele expression ratios in F1 hybrid mice using restriction site polymorphisms which differentiate the 129/Sv Cftr allele from the C57Bl/6J Cftr allele. In F1 mice, $76\% \pm 3\%$ (n=21) of the Cftr mRNA in the trachea is from the 129/Sv allele, while this allele contributes only $35\% \pm 2\%$ (n=25) in the distal colon. The preferential presence of one allele vs. the other must be due to differential allele transcription, pre-mRNA processing, and/or stability of the mRNA. The small variation between samples suggests genetic regulation. Backcross and F2 data from the trachea and distal colon resemble F1 data, suggesting that the role of strain-specific differences in trans-acting factors contribute little to allelic preference, implying a need to investigate cis-acting factors. Preliminary data indicate that while there is no difference in the distal colon, the 129/Sv Cftr mRNA is less stable than the C57Bl/6J Cftr mRNA in the trachea of F1 animals. We have sequenced 1.1Kb of the 1.6Kb 3'UTR from both strains and have identified thirteen single nucleotide polymorphisms. None of these single base changes occur within motifs associated with mRNA degradation. We have also performed initial studies to look at pre-mRNA processing, which indicate that each allele is processed with similar efficiency. Information from this genetic system may further our understanding of the molecular biology of the CFTR gene and its products, ultimately providing insights necessary for effective treatment of CF.

***JAGGED1* transcripts in patients with Alagille syndrome.** C. Crosnier¹, C. Driancourt¹, N. Raynaud¹, M. Hadchouel^{1,2}, M. Meunier-Rotival¹. 1) INSERM U347, Kremlin-Bicetre, France; 2) Service d'Hepatologie Pediatrique, Hopital de Bicetre, Kremlin Bicetre, France.

Heterozygous mutations in *JAGGED1*, encoding a ligand for Notch receptors, cause Alagille syndrome (AGS, MIM 118450), a polymalformative disorder affecting mainly the liver, heart, skeleton, eye and face. Minor features involving kidney, arteries, ear and limb have also been described. We have shown a good correlation between the sites of *JAGGED1* transcription during human development, and tissues affected in the syndrome. Nevertheless, the pathophysiological effect of the mutations is not well defined and expression studies of the modified mRNAs/proteins remain to be performed. We used RT-PCR and sequencing to investigate the presence of mutated mRNA in lymphoblastoid cell lines of 16 patients with mutations identified in DNA (5 missense, 3 nonsense, 6 frameshifts and 2 splice site mutations). mRNAs from 9 patients with no identified mutation were also studied. Primers were designed to amplify the total *JAGGED1* coding sequence in 6 overlapping fragments which were sequenced. The mRNAs carrying missense mutations were roughly as abundant as their wild-type counterpart. The amounts of transcripts with nonsense, frameshift and splice site mutations was more variable, from absence of the modified mRNAs to levels equal to those of the wild-type allele. Mutations were identified in 3 of the 9 patients with no known *JAGGED1* mutation (2 point mutations and a deletion). No mutation was found in 5 other patients after sequencing the coding sequence but heterozygous polymorphisms indicate the presence of transcripts from both alleles. A defect in *JAGGED1* regulatory sequences is thus unlikely. These results suggest that: 1/ mRNA analysis can identify mutations not detected by SSCP when the mutated allele is transcribed; 2/ transcripts with missense mutations are stable but transcripts with mutations causing putative premature stop codons are less, albeit variably abundant, suggesting variable degradation by the nonsense-mediated mRNA decay pathway according to the position of the mutations; 3/ other genes may be responsible for AGS in patients expressing two unaffected *JAGGED1* alleles.

OCA1: Molecular analysis of a complex phenotype. *J.P. Fryer, J.E. Pietsch, S. Ramasubramani, M.J. Brott, W.S. Oetting, R.A. King.* Department of Medicine and Institute of Human Genetics, University of Minnesota, Minneapolis, MN.

Background: Mutations of the tyrosinase gene produce oculocutaneous albinism type 1 (OCA1). The phenotype of this type of albinism is complex and divided into those with no pigment (OCA1A) and those who develop pigment after birth (OCA1B). **Methods:** Using the OCA1 criteria of albinism associated with white hair, white skin and blue irides at birth, we have studied 42 individuals with OCA1A and 21 individuals with OCA1B. DNA was analyzed using automated fluorescent DNA sequencing methods established in our laboratory. Analysis included sequencing each exon, each intron:exon boundary, 500 bp of the 5' promoter region and 90 bp of the 3' untranslated region. **Results:** For individuals who had the phenotype of OCA1A, 29/42 (69 percent) had two mutations identified, 9/42 (21 percent) had only one of two mutations identified, and 4/42 (10 percent) had neither mutation identified. 25/29 (86 percent) were compound heterozygotes with different maternal and paternal mutations. For individuals with the phenotype of OCA1B, 5/21 (24 percent) had two mutations identified. 3/5 were compound heterozygotes and their mutations included T373K, G346X, P406L, IVS2-7, and IVS2+2. 12/21 (57 percent) were compound heterozygotes with only one identified mutation, and 4/21 (19 percent) had neither mutation identified. The OCA1B phenotype ranged from minimal to nearly normal cutaneous pigmentation. Southern analysis was normal for those with one or no mutation identified. The normal allele was visualized for all compound heterozygotes in whom only one mutation was identified (no deletion). No mutations were found in the promoter region of the gene. **Conclusion:** Analysis of the coding region of the tyrosinase gene identifies only 56 percent of the mutations responsible for inactivation of this key pigment enzyme. For OCA1B, the majority (57 percent) of individuals have only one of two mutations identified. These studies suggest that there are additional key regulatory regions of the tyrosinase gene beyond the proximal promoter region, or other non-coding regions where mutations can have a profound effect on the production of active enzyme.

Heterozygous mutations in *CDMP1* cause a spectrum of skeletal phenotypes through different effects on protein production and function. *D.B. Everman*¹, *N.H. Robin*¹, *J. Marcelino*¹, *J.T. Thomas*², *J.T. Hecht*³, *M.L. Warman*¹. 1) Dept. of Genetics, Case Western Reserve Univ., Cleveland, OH; 2) National Institutes of Health, Bethesda, MD; 3) Univ. of Texas, Houston, TX.

Cartilage derived morphogenetic protein 1 (CDMP1) is a secreted signaling molecule important to the development of bones and joints. Like other members of the TGF- β superfamily, CDMP1 is synthesized as a larger precursor that undergoes dimerization and proteolytic cleavage to generate a mature signaling molecule. A conserved RRRRR amino acid sequence comprises CDMP1's proteolytic cleavage site. Heterozygous frameshift and nonsense mutations in *CDMP1* cause autosomal dominant brachydactyly type C (BDC), implying that BDC results from functional haploinsufficiency. Interestingly, missense mutations in *CDMP1* have been associated with other skeletal phenotypes. We have observed an Arg380Trp mutation that alters the conserved cleavage site in two families with fibular hypoplasia and BDC, while a Ser475Asn mutation has been reported in a family with multiple synostoses syndrome type 2 (SYNS2).

To determine how these mutations alter CDMP1 function, we expressed wild-type and mutant CDMP1 in COS-7 cells and studied protein production, dimerization, proteolytic cleavage, secretion, and interaction with noggin, a CDMP1 binding partner that when mutated causes multiple synostoses syndrome type 1. We have found that the Arg380Trp mutation impairs proteolytic cleavage of dimeric CDMP1, causing markedly reduced secretion of the mature signaling molecule. Cleavage is impaired in both mutant homodimers and mutant/wild-type heterodimers, suggesting that the mutation acts via a dominant negative mechanism and explaining why it causes a more severe phenotype. In contrast, the Ser475Asn mutation does not adversely affect CDMP1 synthesis, processing, secretion, or noggin binding. This suggests that the mechanism by which this mutation causes SYNS2 may involve downstream activities of CDMP1 such as receptor binding and activation. These results provide insight into the mechanisms by which heterozygous CDMP1 mutations cause a wide variety of skeletal phenotypes.

Evidence that human frataxin is assembled in a large multimeric complex in human mitochondria. R. Airoidi¹, V. Seveso¹, C. Gellera¹, D. Barisani², F. Taroni¹. 1) Istituto Nazionale Neurologico "C. Besta", Milan, Italy; 2) University of Milan, Milan, Italy.

Friedreich's ataxia (FRDA), the most common hereditary ataxia, is caused by a deficiency of frataxin, a 210-aa nuclear-encoded mitochondrial protein. Studies on yeast mutants lacking the yeast frataxin homologue Yfh1p have suggested that frataxin may play a critical role in mitochondrial iron homeostasis and free radical toxicity. Although several observations indicate that human frataxin may have a similar role, its function still remains elusive. Human mature frataxin is a 155-aa protein with a predicted molecular mass of 17,257 Da (Cavadini, 2000). It has recently been reported that purified mature Yfh1p may assemble *in vitro* in a macromolecular spherical complex of 60 subunits and 1.1 MDa, following the aerobic addition of ferrous ion (Isaya, ASHG1999). These higher order multimers can sequester up to 69 Fe atoms per subunit maintaining Fe in a soluble, available, and non-toxic form. We have investigated the presence of similar macromolecular complexes in human cells by using 2D PAGE. To obtain highly specific and sensitive anti-frataxin Abs, a GST-frataxin fusion protein was expressed in *E. coli* and injected into rabbits. Cultured human cells were permeabilized with digitonin and proteins from the resulting mitochondria-enriched fractions were first separated on a 5-15% polyacrylamide gradient gel under non-denaturing conditions (1st D, Blue Native PAGE). In the 2nd D, the macromolecular protein complexes were further resolved into their individual subunits by denaturing SDS-PAGE. Probing with anti-frataxin Abs clearly demonstrated the presence of cross-reacting material of molecular weight >200 kDa in cultured lymphoblasts, HeLa, and human hepatoma HepG2 cells. The signal was significantly reduced in FRDA lymphoblasts and increased in HeLa cells overexpressing a transgene encoding human frataxin. These results support the proposed hypothesis (Isaya, ASHG1999) that frataxin may act as an iron storage protein and, similar to cytoplasmic ferritin, may play a role in iron detoxification and iron reserve in the mitochondrion. (Supported by a Telethon-Italia grant to FT).

Identification of novel sequence variants in the NF1 promoter region: mutational and functional analysis. *M.P. Horan, M. Upadhyaya.* Inst Medical Genetics, Univ Wales Col Medicine, Cardiff, Wales.

Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder characterised by multiple café-au-lait spots and multiple neurofibromas. The NF1 patient associated mutational spectrum for the 60 exon NF1 gene is now well established, and there is no evidence of any mutational clustering. To-date, there is no report of any disease-associated NF1 promoter mutations. The NF1 gene contains at least 22 putative transcription factor binding sites, and it is important to ascertain whether sequence variants within the regulatory control regions of the NF1 gene have any effect on the level of protein expression. Using a combined single stranded conformational polymorphism and heteroduplex approach, a 1kb region which encompasses the transcription initiation site of the NF1 gene was analysed in 570 unrelated NF1 patients and 105 normal subjects. Three novel polymorphisms were identified at positions -142, +261, +462 relative to the transcription initiation site, a 14bp deletion and two nucleotide substitution (C→G and G→C). Electromobility shift assays identified extra proteins binding to these 3 polymorphic sequences. The polymorphic sequence at position -142 bound 3 additional protein bands, while nucleotide sequences at positions +261 and +462 each bound an extra protein each. A Transcription Factor search programme was used to compare and contrast the putative transcription factor binding motifs associated with both the normal and variant sequences. The deletion polymorphism was predicted to result in the loss of 20 putative transcription factors, the substitution at +261 apparently had no effect, while the substitution at position +462 was predicted to result in an increase of 17 transcription factors which putatively bind to this region. Functional analysis of the alterations at -142 and +261 in HeLa and cerebellum TE61 cells, failed to reveal any differences in comparison to the wild type sequence. A slight decrease in reporter gene activity was observed with the construct harboring the G→C change at +462. This study indicates that these polymorphic variants are unlikely to be of immediate mutational importance in the development of the NF1 phenotype.

Heterodimerization between wild-type and mutant TIGR/myocilin polypeptides is critical for autosomal dominant open-angle glaucoma. V. Raymond¹, M.A. Rodrigue¹, S. Gobeil¹, I. Fleury¹, S. Moisan¹, T.D. Nguyen², J.R. Polansky², J. Morissette¹. 1) Molecular Endocrinology, Laval University Hospital (CHUL) Research Ctr, Québec City, PQ, Canada; 2) Ophthalmology, UCSF, San Francisco, CA.

Mutations in the *trabecular meshwork-induced glucocorticoid response (TIGR)/myocilin* gene cause autosomal dominant open-angle glaucoma, a disorder characterized by an optic neuropathy often associated with ocular hypertension. One of these variants: *TIGR*^{K423E}, manifests autosomal heterozygote-specific dominance (AHSD). To understand the mechanisms by which these mutations cause glaucoma, we investigated the structure and properties of TIGR polypeptides. COS-7 and human trabecular meshwork (HTM) cell lines were transfected with expression vectors encoding *TIGR* wild-type (wt), mutated and/or epitope tagged cDNAs. TIGR polypeptides were analyzed by immunoblotting. Our studies showed that all cDNAs generated 2 isoforms, 1 of which was N-glycosylated at Asn-57. Under non-reducing conditions, *TIGR*^{wt} formed 2 major intracellular complexes migrating at about 120 and 240 kDa. The 120 kDa complex resulted from TIGR homodimerization. When transfected alone, the G364V, Q368X, K423E and Y437H disease-causing mutants also formed homodimers. Co-transfection of mutant cDNAs with *TIGR*^{wt} produced wt/mutant heterodimers. *TIGR*^{wt} homodimers were secreted into COS-7 and HTM culture media. TIGR homodimers were also observed in human aqueous humor. Disease-causing mutant homodimers, however, were not detected outside the cells. Transfections of increasing amounts of mutant cDNAs with decreasing concentrations of *TIGR*^{wt} revealed that wt/mutant heterodimers also remained sequestered within intracellular compartments. These data suggest that heterodimerization between wt and mutant TIGR/myocilin polypeptides may play a critical role in causing glaucoma probably by hampering TIGR extracellular functions required for normal aqueous outflow and/or by increasing the amount of intracellular wt/mutant heterodimers which may then interfere with HTM cell functions. Our experiments also suggest that several *TIGR* mutations may act in a dominant negative fashion and support metabolic interference to account for AHSD of glaucoma.

Functional analysis of the putative peroxidase domain of FANCA, the Fanconi anemia complementation group A protein. *J. Ren, H. Youssoufian.* Dept. Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Fanconi Anemia (FA) is an autosomal recessive disorder manifested by chromosomal breakage, birth defects, and susceptibility to bone marrow failure and cancer. There are at least eight complementation groups of FA, and the genes defective in five groups have been cloned. Worldwide, complementation group A is the most common subtype. Although we and others have shown that the FA group A protein (FANCA) and the group G protein (FANCG) interact with each other in the nucleus, these proteins have no homology to each other and their normal function is obscure. A clue to the function of the FANCA protein was recently provided by analysis of its coding sequence and the detection of limited homology in the amino terminal region to a class of heme peroxidases. We evaluated the functional importance of these conserved residues by mutagenesis and cDNA complementation studies. We substituted alanine residues for the most conserved FANCA residues in the putative peroxidase domain: His184-to-Ala, Asp237-to-Ala, His305-to-Ala, and Trp183-to-Ala. While all of the substitution mutants were comparable to wild type FANCA with regard to their stability, subcellular localization, and interaction with FANCG, only the Trp183-to-Ala substitution abolished the ability of FANCA to complement the sensitivity of FA group A cells to mitomycin C. There were no differences between parental FA group A cells and cDNA complemented cells in their sensitivity to hydrogen peroxide. Thus, Trp183 is essential for the *in vivo* activity of FANCA. However, these results do not support the hypothesis that FANCA has peroxidase activity.

DGGE scan as a tool to look for new mutants and carriers of the DMD gene. *L.C. Dolinsky, R.S. Moura-Neto, D.N. Falcão-Conceição.* Dept of Genetics, Inst de Biologia, Univ Fed do Rio de Janeiro, Rio de Janeiro, R.J., Brazil.

Approximately 30% of Duchenne muscular dystrophy(DMD) patients have undefined mutations in the dystrophin gene, face the difficulty to identify single nucleotide variations in genomic DNA using current diagnostic techniques. It represents a great obstacle in genetic analysis of these patients and genetic counseling of their families. In this work, we performed a denaturing gradient gel electrophoresis(DGGE)to search for single nucleotide variations. DGGE has a proportion of detectable mutations higher than others techniques, such as single strand conformation polymorphism and heteroduplex analysis. Our goal here is to know if DGGE technique is a good approach to look for new mutants and carriers of the DMD gene. In our data 60% of Brazilian DMD patients, that do not have a detectable deletion or duplication, are isolated cases, i.e. there is no family history of the disease. The genetic counseling for these families is really difficult once we do not know whether the patient is a new mutant or the mother patient already has the DMD mutation. We studied 20 mothers of Brazilian DMD isolated patients, that do not have a detectable deletion or duplication. We performed DGGE analysis for the mothers and their sons and once we found a shift, we sequenced the PCR product to know the exact disease causing mutation. So far we studied 70% of the DMD gene and we could identify in 15 families(75%) whether the patient is a new mutant or the mother was already a carrier of the DMD gene. All but one mutation we could identify here were not described before. We observed that in only three cases(3/20) the patients were new mutants. In 60% of the families(12/20), the mutation was already present in the mother, that corroborate previous studies about grand-paternal origin of small mutations. We conclude that DGGE is a very efficient method to look for new mutants and carriers of the DMD gene in the 30% of patients that have single nucleotide variations. DGGE showed high mutation detection rate(close to 100%) and can be used as a current diagnostic procedure to improve genetic analysis and counseling.(Supported by CAPES, FUJB, CNPq and Prinses Beatrix Fonds).

FSHD myoblasts possess reduced resistance to oxidative stress. *K.A. Barrett^{1,2}, R. Tawil², R.C. Griggs¹, D.A. Figlewicz^{1,2}.* 1) Dept Neurobiology & Anatomy, Univ Rochester, Rochester, NY; 2) Dept Neurology, Univ Rochester, Rochester, NY.

Facioscapulohumeral muscular dystrophy(FSHD), the third most common muscular dystrophy, is inherited in an autosomal dominant manner. A variable deletion in a repeat region (D4Z4) of chromosome 4q35 has been associated with the disorder, however the pathogenesis of FSHD has yet to be established. Myoblasts from FSHD patients possess a necrotic appearing morphology, with a swollen cytoplasm and perinuclear vacuoles. Highly confluent cells appear to lack the organization of normal myoblasts, and FSHD cells fuse to form disorganized, swollen myotubules. The predominance of the necrotic phenotype may be relatively specific to FSHD as cells from other muscle diseases (myotonic dystrophy, Becker myotonia, desmin storage myopathy) exhibit a morphology indistinct from normals. Because exposure to the superoxide anion generator, paraquat, induced a similar phenotype in normal myoblasts, we studied the survival of FSHD, normal and other muscle disease myoblasts and myotubules after exposure to an oxidative stressor (paraquat) and a non-oxidative stressor, the protein kinase C inhibitor, staurosporine. The only statistically significant difference was observed for FSHD myoblasts exposed to low concentrations of paraquat (0.02 mM and 0.2 mM). FSHD myoblasts showed decreased survival rates relative to normal and disease controls. Work by others has shown upregulation of the cdk inhibitor, p21, in circumstances of oxidative stress in fibroblasts. We have found that FSHD myoblasts under normal growth conditions express higher baseline levels of p21 compared to normal control cells (22.2% of FSHD myoblast nuclei stain strongly positive for p21 compared to 14.2% of normal myoblast nuclei, $p=0.004$, $n=4$). The current study demonstrates an enhanced vulnerability of FSHD myoblasts to oxidative stress, suggesting a biochemical marker for FSHD early in myocyte development.

A missense-mutation (Glu706Lys) in the skeletal myosin heavy chain type 2A gene causes a muscle fiber type specific congenital myopathy. *T. Martinsson*¹, *A. Oldfors*², *N. Darin*³, *K. Berg*¹, *H. Tajsharghi*², *M. Kyllerman*³, *J. Wahlstrom*¹. 1) Departments of Clinical Genetics; 2) Pathology; 3) Pediatrics, Sahlgrenska Univ Hosp, Gothenburg University, Gothenburg, Sweden.

Myosin is an actin-based molecular motor protein that transduces chemical energy of ATP hydrolysis into mechanical force. Several genes encode myosin heavy chains (MyHC) in striated muscle of mammals. Alfa and beta MyHC are encoded on chromosome 14, while the genes encoding embryonic, Ila, IIX/d, IIB, perinatal, and extraocular MyHCs are located in a cluster on chromosome 17. Beta myosin is the main constituent of cardiac myosin and numerous point mutations in the beta MyHC gene have been reported in association with familial hypertrophic cardiomyopathy. We have previously described in a family an 'autosomal dominant myopathy, with joint contractures, ophthalmoplegia, and rimmed vacuoles', a variant of hereditary inclusion body myopathy (hIBM). Linkage analysis and radiation hybrid mapping showed that the gene locus (HGM locus name: IBM3) is situated in a 2 Mb region of chromosome 17p13, where also the cluster of MyHC genes is located. Analysis of muscle biopsies from patients from the family indicated to us that the type 2A fibers frequently were abnormal, while other fiber types appeared normal. This prompted us to investigate the MyHC-Ila gene and the complete genomic sequence for this gene was deduced using an "in silico" strategy. The gene, found to consist of 38 exons, was subjected to a complete mutation scan in patients and controls. We identified a missense mutation, Glu706Lys, which is located in a highly conserved region of the motor domain, the so-called SH1 helix region. By conformational changes this region communicates activity at the nucleotide binding site to the neck region, resulting in the lever arm swing. The mutation in this region is likely to result in a dysfunctional myosin, compatible with the disorder in the family. This is the first human myopathy found to be associated with a mutation in a fast myosin gene, and the first identified genetic defect in hIBM.

Expression profiling in the muscular dystrophies uncovers novel features underlying progressive pathophysiology. *Y.-W. Chen, P. Zhao, R. Borup, E.P. Hoffman.* Research Center for Genetic Medicine, CNMC, Washington, DC.

We tested the hypothesis that comparison of genome-wide expression profiling in two closely related biochemical defects, dystrophin-deficiency (Duchenne muscular dystrophy), and alpha-sarcoglycan deficiency would define novel aspects causing the progressive pathophysiology of these muscular dystrophies. Further, we tested the hypothesis that any clear differences in diff-calls between patients with primary deficiencies of these neighboring proteins are biochemical partners. We studied muscle biopsies from 5 patients with dystrophin-deficiency, 4 alpha-sarcoglycan deficiency, and 5 age-matched male controls. Five biopsies were split, and processed in parallel, with biotinylated cRNA mixed into equimolar pools. Duplicate pools were hybridized to Affymetrix HuGeneFL GeneChips. We found a constant level of 33% present calls in all muscle biopsy samples, with approximately 20% of them showing 2-fold or greater differences in expression between dystrophic and normal muscle (495 diff-calls DMD/normal; 407 diff-calls alpha-sarcoglycan/normal). Diff-calls held in common between the two dystrophies showed many shared gene expression changes that fell into specific pathophysiological cascades. Down-regulated clusters were wide-ranging decreased in genes associated with energy metabolism (both mitochondrial, and glycogenesis), which are explained by decreased expression of key regulatory genes in known pathways. Upregulated clusters were more complex, with dramatic increases in connective tissue components (42%), immune responses (20%), and cell growth and differentiation (15%). As an extension of expression array analyses, we tested the hypothesis that detection of dramatic gene expression changes in dystrophin-deficiency, which were not shared with alpha-sarcoglycan deficiency, would identify dystrophin-associated proteins. Indeed, we found that ERK6 was 10-fold underexpressed specifically in dystrophin-deficiency; this protein has very recently been found to interact with alpha-syntrophin. In addition, a novel protein phosphatase (PTPH1) was similarly decreased, suggesting that this also may be involved in dystrophin-mediated signaling pathways.

A novel intermediate filament protein that interacts with alpha-dystrobrevin 1. *Y. Mizuno¹, T.G. Thompson¹, H.G.W. Lidov¹, M. Brosius¹, M. Imamura², E. Ozawa², L.M. Kunkel¹.* 1) Howard Hughes Medical Institute and Division of Genetics, Childrens Hospital, Boston, Massachusetts 02115, USA; 2) Division of Cell Biology, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Tokyo, 187-8502, Japan.

Alpha-dystrobrevin 1 is a member of the dystrophin-associated protein complex (DAPC) that directly interacts with dystrophin, α 1-syntrophin and sarcoglycan complex although the precise role of α -dystrobrevin 1 in skeletal muscle has not been determined. To further study α -dystrobrevin 1 and the role it plays in maintaining muscle integrity, we looked for new interacting proteins. Using the yeast two-hybrid system, we tested exons 1 through 16 of α -dystrobrevin 1 and identified three overlapping clones representing a new intermediate filament protein, which we term DIP-1 (dystrobrevin-interacting protein 1). Full length DIP-1 was obtained by screening a skeletal muscle cDNA library and 5' RACE. By northern blot analysis, DIP-1 is mainly expressed in cardiac and skeletal muscles although there is a small amount in brain. Interestingly, by western blot analysis, DIP-1 was found to be a 150 kDa protein in cardiac and skeletal muscles but was not found in brain. Through immunohistochemistry analysis of adult human skeletal muscle, DIP-1 appears to be located within the contractile apparatus in a stripe-like pattern. The interaction between DIP-1 and α -dystrobrevin 1 was confirmed and a more precise area of interaction determined using in vitro coimmunoprecipitation. This region includes exons 8 through 16 of α -dystrobrevin 1 and the central alpha helical domain of DIP-1 as determined by sequence comparison to other intermediate filament proteins. The finding of an interaction between a novel intermediate filament protein and α -dystrobrevin 1 suggests that the DAPC interacts with the cytoskeleton through several proteins in addition to dystrophin.

Differential gene expression analysis in Emery-Dreifuss muscular dystrophy using cDNA microarrays.. *T. Tsukahara, S. Tsujino, M. Ogawa, A. Nishiyama, M. Fujita, R. Kurokawa, Y.K. Hayashi, K. Arahata.* Dept Neuromuscular Research, Natl Inst Neuroscience, NCNP, Tokyo, Japan.

Emery-Dreifuss muscular dystrophy (EDMD) is an inherited muscular disorder. Most patients show X-linked recessive inheritance (X-EDMD), but a few autosomal dominant form (AD-EDMD) and rare autosomal recessive form (AR-EDMD) have been reported. The gene products for X-EDMD (emerin) and AD-EDMD (lamin A/C) have been localized to the nuclear envelope (Nagano et al, 1996, Manilal et al, 1996, Mora et al., 1997, Bonne et al, 1999), thus EDMD is the first muscular dystrophy found to be caused by mutations of genes for the nuclear envelope proteins. To clarify the molecular mechanism of X-EDMD, we monitored the gene expression patterns in cultured fibroblasts from normal controls and three patients with genetically confirmed X-EDMD using cDNA microarrays. NEN Micromax human cDNA microarray system I was used, which contains 2400 known human genes. Four ug of total RNA was analyzed for both DNP-Cy3 and Biotin-Cy5 system, and the ScanArray 5000 measured the fluorescent signaling. A total of 63 genes showed changes ($>2x$ at least in a patient and $>2\sim 1.5x$ in two patients) in expression in X-EDMD (42; upregulated, 22; down regulated). Lamin A/C gene was upregulated in all three patients fibroblasts. To know if these changes are due to primary emerin deficiency, we tried to restore the gene expression by a transfection of the gene-therapeutical adenoviral vector for human emerin gene (AxCAN-Eme). AxCAN-LacZ was used for control. We found that 32 of the 63 genes, including lamin A/C gene, were restored by the AxCAN-Eme transfection. Our results implicate the crucial roles for the nuclear envelope proteins, emerin and lamin A/C, in the development of X-EDMD, and are the first step in the elucidation of the pathogenesis of EDMD.

Expression profiling of 4q35/10q26 transcripts in FSHD using cDNA microarrays. *S.T. Winokur¹, P.S. Masny¹, S. van der Maarel², D.A. Figlewicz³, K. Arahata⁴, K. Flanigan⁵.* 1) Biological Chemistry, University of California, Irvine, CA; 2) Human Genetics, Leiden University Medical Center, Netherlands; 3) Neurobiology and Anatomy, University of Rochester, NY; 4) National Institute of Neuroscience, Tokyo, Japan; 5) Eccles Institute of Genetics, University of Utah, Salt Lake City, UT.

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant, progressive neuromuscular disorder. The disease arises from an unusual genetic mechanism, a deletion of 3.3 kb tandem repeats in the subtelomeric region of chromosome 4q (distal 4q35). On the normal chromosome 4, these repeats (D4Z4) exhibit many characteristics of heterochromatin and are highly methylated. A homologous region is present in the subtelomeric region of chromosome 10 (distal 10q26). FSHD is believed to result from a position effect, in which disruption of the normal chromatin structure at 4qter affects the expression of genes proximal to D4Z4. We have therefore generated a custom cDNA microarray to address the region-wide expression of genes in FSHD. Fifty-two (52) genes and ESTs from 4q35 and fifteen (15) genes from 10q26 were amplified and spotted onto amino-silane coated glass slides. Initial studies examined the relative expression levels in both adult skeletal muscle and fetal brain RNA. One ug of total RNA was labelled with Cy5 (skeletal muscle) or Cy3 (fetal brain). Thirty-five (35) transcripts in the FSHD region (4q35) and 9 genes from 10q26 are expressed at some level in normal skeletal muscle, with thirteen (13) of these being predominantly expressed only in the skeletal muscle sample. In order to examine differential gene expression in FSHD, we have isolated RNA from FSHD and control muscle biopsies. Matched biopsies (i.e. unaffected and affected muscle) from four FSHD patients were chosen in order to eliminate "noise" due to human variability. Relative quantitation for each of these 4q35/10q26 transcripts was determined in FSHD, normal and disease control muscle. In addition, myoblast cell lines established from FSHD and normal muscle were used for expression analysis of these transcripts through the process of muscle differentiation.

Validation of expression profiling: effect of multiple tests, and use of antibody confirmation on patient tissues

with known genetic defect. *P. Zhao*¹, *Y-W. Chen*¹, *F. Vivanco*², *J. Lawler*³, *E.P. Hoffman*¹. 1) Research Center for Genetic Medicine, CNMC, Washington DC; 2) Immunology, Clinica Coucepcion, Madrid, Spain; 3) Pathology, Beth Israel Deaconess Medical Center, Boston, MA.

Expression profiling of RNA in patient tissue promises to rapidly advance knowledge of human disease cause and pathophysiology. Use of affected patient tissues introduces variables which must be considered in experimental design, such as background genetic differences, heterogeneity in tissue sample, and many others. Here, we present a protocol for minimizing these variables using patient tissue samples, while maximizing the information content (diff calls). To control for tissue heterogeneity, each patient muscle biopsy is divided in half, and processed for RNA and biotinylated cRNA in duplicate. To control for background genetic differences, mixing experiments were conducted using 5 DMD biopsies, and 5 sex- and age-matched normal controls, which are then mixed into equimolar pools prior to hybridization to Affymetrix HuFL GeneChips. This provides two datafiles of expression data for DMD, and two for controls, from different regions of the same biopsies. Comparison of datasets showed that only 30-40% of diff calls are able to survive four iterative comparisons of datasets. This shows that conduct of multiple experiments are critical before analyzing diff calls. We then selected a series of diff calls for verification by either TaqMan quantitative RT-PCR, or immunofluorescence on patient muscle biopsies. We found TaqMan verification to be cumbersome and expensive. Verification at the protein level was rapid and cost-effective, providing valuable additional information on the cell-type expression and localization of the diff call gene product. Novel aspects of DMD pathogenesis identified included dramatic infiltration of dendritic cells (HLA-DR+, Factor XIII+). Novel components of endomysial fibrotic replacement of DMD muscle (SPARC/osteonectin, thrombospondin-4) were identified. Finally, we show that necrotic myofibers express extremely high levels of Complement component 3 and phospholipase A2. In all, 11 antibodies were tested, and in all cases the protein results confirmed the GeneChip data.

Molecular analysis of phenylketonuria (PKU) in newborns from Texas. *J. Garcia-Heras¹, Y. Yang¹, L.M.*

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We describe the spectrum of mutations at the phenylalanine hydroxylase (PAH) locus in patients with classical PKU, non-PKU hyperphenylalaninemia (HPA) variant and benign persistent hyperphenylalaninemia (HPA) variant ascertained by the Texas Newborn Screening Program.

Blinded DNA studies were done in 40 cases (16 of classical PKU, 12 of non-PKU HPA variant and 10 with benign persistent HPA). Twenty four patients were white, twelve hispanic, 1 black and in 3 the race or ethnic origin was unknown. Genomic DNA was isolated from dry blood spots (Guthrie cards). The 13 exons of the PAH gene were amplified by PCR using specific primers and analyzed by automatic DNA sequencing.

Thirty six different mutations were detected in 75/80 mutant chromosomes (diagnostic efficiency of 94%). There were 22 missense mutations, 6 splice mutations, 2 nonsense mutations and 3 deletions. The prevalent mutations were R408W (15/80), Y414C (5/80), IVS10nt-11g->a (5/80), H170D (4/80), A403V (3/80), V388M (3/80), T380M (3/80) and IVS7nt1g->a (3/80). There were 3 previously unreported missense mutations, all in exon 5 of the PAH gene: H170D (5/80), N167S (1/80) and R169H (1/80). The other 25 mutations had much lower frequencies (1-2/80).

There was genotype/phenotype correlation in more than 70% of cases.

The preliminary data on this population suggest that exons 12, 7, 11, 5, and 6 harbor most mutations (64%) and should be screened first. But, when only one or no mutations are identified in this first molecular screening all the other exons must be studied.

This study will continue collecting data about the mutations associated with PKU and hyperphenylalaninemia in the newborns of Texas. Of special interest are the Hispanics who have not been studied extensively and are descendants from populations carrying mutant alleles different from Northern Europe or other ethnic origin.

Frequencies of Mutations in the SLC7A9 and the SLC3A1 genes in unclassified cystinuric patients. T.

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Cystinuria is a recessive disorder of renal reabsorption of cystine and the dibasic amino acids. Mutations in the SLC3A1 gene encoding the glycoprotein rBAT cause type I cystinuria. Recently, the b0,+AT protein has been identified as a light subunit of rBAT. Mutations in its gene SLC7A9 were demonstrated in non-type I cystinuric patients. We searched for variants in SLC7A9 and SLC3A1. Our study population consists of 18 unclassified and 5 type I cystinurics. Mutation analysis was performed by SSCP, restriction assays and direct sequencing. In the unclassified patients, point mutations in SLC3A1 were detected in 20 chromosomes, corresponding to a frequency of nearly 56%. The most frequent variants were T216M in Greek and M467T in German patients. Screening of the SLC7A9 gene revealed 4 mutations, therefore corresponding to a frequency of 11%. Out of these, three have been described before, additionally we detected a new 3 bp-deletion (DE244). Furthermore, 4 new polymorphisms were identified. One of our patients showed compound heterozygosity for G105R and DE244, another showed mixed heterozygosity for M467T in SLC3A1 and G105R in SLC7A9. In type I patients, mutations were restricted to SLC3A1. In total, the higher mutation detection rate in SLC3A1 corresponds to the clinical finding that type I is more frequent than non-type I cystinuria. A detection rate of 66% for mutations in both genes can be delineated. Large deletions as described in SLC3A1 as well as mutations in non-coding regions were not considered, thus an increased detection rate can be expected. Nevertheless, as demonstrated for cystinuria type I and the SLC3A1 gene, further factors should be involved in the pathogenesis of the disease. Mutations in these genes might cause the phenotypic features in those patients lacking mutations in SLC3A1 and SLC7A9. Acknowledgement: Primers and genomic data were provided by the International Cystinuria Consortium.

Spectrum of mutations in the NPHS1 gene in non-Finnish patients with congenital nephrotic syndrom. O.

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Congenital nephrotic syndrome is a recessively inherited disease characterized by massive proteinuria already *in utero*, and nephrotic syndrome shortly after birth. The disease is most common in Finland (congenital nephrotic syndrome of the Finnish type, CNF) and belongs to the Finnish disease inheritance. Recently, the NPHS1 gene, coding for the novel kidney specific transmembrane protein nephrin, was cloned in our laboratory. Consequently, several mutations were identified in the NPHS1 gene in patients with congenital nephrotic syndrome. The NPHS1 gene contains 29 exons. In Finnish population, two main mutations have been found. Both of them lead to synthesis of truncated protein. Fin_{major} is a two-base-pair frameshift deletion in exon 2 and Fin_{minor} is a nonsense mutation in exon 26. The main Finnish mutation, Fin_{major}, is estimated to account for about 78% and Fin_{minor} about 16% of the affected Finnish chromosomes.

In this study, screening of NPHS1 mutations using PCR and direct sequencing was performed in non-Finnish patients with congenital nephrotic syndrome. Patient samples were obtained from Sweden, Germany, France, UK, Turkey and USA. Both Fin_{major} and Fin_{minor} were identified in the foreign samples but they accounted only for a small part of the cases. The other mutations included splice site, missense and nonsense mutations together with deletions and insertions (in-frame and frameshift). In most cases we found "individual" mutations, but some were found in more than one patient. The cloning and identification of mutations in the NPHS1 gene enable us to perform exact DNA-based prenatal diagnoses for parents of non-Finnish origin.

Rapid detection of genomic rearrangements using Multiplex PCR of Fluorescent Fragments. *T. Frebourg¹, C. Di Rocco², F. Charbonnier¹, N. Drouot¹, S. Mazoyer⁴, D. Stoppa-Lyonnet³, M. Tosi¹.* 1) INSERM EMI 9906 IFRMP, Faculté de Médecine et de Pharmacie, 76183 Rouen; 2) Unité d'Immunogénétique Humaine, Institut Pasteur, 75724 Paris; 3) Service de Génétique Oncologique, Institut Curie, 75248 Paris; 4) Laboratoire de Génétique, Faculté de Médecine, 69373 Lyon Cedex, France.

Genomic rearrangements such as exon deletions or duplications represent a major class of molecular alterations which cannot be detected by conventional PCR-based methods. Their detection requires procedures, such as long range PCR or Southern-blot hybridization, that are inaccurate, lengthy or require large amounts of high quality DNA. We have optimized a multiplex PCR-based assay that we had initially developed for the detection of mismatch repair gene rearrangements in hereditary non polyposis colorectal cancer (Charbonnier et al. Cancer Research, 2000; 60: 2760-2763). This assay is based on (i) the amplification of fragments covering all exons using dye-labeled primers, (ii) a limited number of PCR cycles in order to keep PCR amplification within the exponential range, and (iii) the superimposition of the peaks generated from different samples after electrophoresis on an automated DNA sequencer. Amplification of numerous fragments, including exon fragments from other genes used as controls, allowed an accurate comparison of different electropherograms. Comparing the heights of the corresponding peaks generated from different samples is more informative than calculating ratios of the different peak areas within the same sample, followed by comparison of these ratios between samples, as proposed in other multiplex PCR-based methods. This method allowed us to identify several new genomic rearrangements of *MSH2* and was shown to be a powerful method for the detection of deletions or duplications of other genes, such as *C1 INH*, *SMN*, or *BRCA1*.

Mutation Analysis, Genotype - Phenotype Correlation and Carrier Frequency of FMF in the Jordanian

Population. *H.E. El-Shanti*^{1,3}, *M. Al-Khateeb*², *M. Tayeh*³, *Z. Abu-Rubaiha*², *A. Pelet*³, *A. Munnich*³, *S. Lyonnet*³, *H.A. Majeed*². 1) Jordan University of Science & Technology, Irbid, Jordan; 2) Jordan University, Amman, Jordan; 3) INSERM U393, Hopital Necker-Enfants Malades, Paris, France.

Familial Mediterranean Fever (FMF) is an autosomal recessive disorder affecting mainly Arabs, Jews, Armenians and Turks. The disorder is characterized by recurrent short episodes of inflammation manifested as fever and pain. The gene responsible for FMF (MEFV) has been cloned and over 25 different mutations have been identified so far. We aimed at identifying the spectrum of MEFV mutations amongst Jordanians, examining correlations between genotypes and disease phenotype, as well as, calculating carrier frequency in the population. We identified a cohort of 37 families, 32 of whom were ascertained through a single proband. The remaining 5 families are complex, with affected individuals in multiple sibships and consecutive generations. We screened for mutations by direct sequencing of the entire exon 10 and its splice sites, and by restriction endonuclease testing for mutations in exon 2. We studied the correlation between the identified genotypes in affected individuals and the disease severity using a severity index, modified from the Tel Hashomere severity scale. We then screened the DNA from a panel of 400 anonymous controls for the 4 most common mutations by ARMS and primer-mediated restriction endonuclease testing to estimate carrier frequency. Out of the expected 64 mutant chromosomes in the one-proband families, 33 mutations (50%) were identified. The mutations M694V and V726A were the most common with almost equal frequency (35% each). The M694I and M680I mutations were present in 20% and 10% of mutant FMF chromosomes, respectively. The pattern of mutations in 3 out of the 5 remaining families was partially solved. In one family, a complex allele of E148Q and M694V was identified. Although, the size of the sample is small to draw firm conclusions, there was a correlation between genotype and the severity of the disease but not between genotype and amyloidosis. Finally, the carrier frequency for the 4 most common mutations was about 10 % in healthy Jordanian individuals.

Involvement of the IL-12 heterodimer in severe idiopathic mycobacterial infections. *H. Elloumi¹, M.R.*

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Interleukin 12 is a 75-kDa heterodimeric cytokine comprised of two disulfide-bonded glycoprotein subunits, p35 and p40. It is primarily produced by macrophages and dendritic cells, and is known to be active early in innate and adaptive immunologic responses to bacterial and parasitic infections. IL-12 has pleiotropic effects on T and NK cells, including the induction of IFN- γ secretion, the stimulation of cell proliferation and the promotion of a Th1 type response. A mutation in IL-12p40 subunit gene has been reported in patient with BCG and Salmonella enteritidis disseminated infection. In the present study, we have screened IL-12 p40 and IL-12p35 genes in three patients presenting BCG disseminated infections. In one patient born to first degree consanguineous parents, we have identified an 8 bp deletion within IL-12p40 gene between positions 297 and 304. This mutation leads to a frameshift that introduces a premature stop codon at nucleotide position 342 to 344 (TGA). This deletion was found in the genomic DNA, the parents are heterozygous for the mutation. The two other patients are siblings born to unrelated parents. Mutations in IL-12p40 gene as well as in IFN γ R1, IFN γ R2, IL-12R β 1 genes have been excluded by sequencing. PCR amplification and sequencing of IL-12p35 cDNA allowed the identification of a 146 nucleotides deletion resulting from a splice mutation. This is the first report of a mutation in the IL-12p35 gene causing disseminated infections due to (BCG).

New splice mutations within the IL-12Rb1 gene causing severe mycobacterial infections. *R.M. Barbouche¹, H. Elloumi¹, J. Chamli², M. Béjaoui³, B. Larguèche¹, S. Abdelhak¹, K. Dellagi¹.* 1) Immunology Department, Pasteur Institute, Tunis, Tunisia; 2) Pediatric Department, Sahloul Hospital, Sousse, Tunisia; 3) Bone Marrow Transplantation Center, Tunis, Tunisia.

The immunoregulatory cytokine IL-12 plays a central role in cell mediated immune responses through interaction with cell surface receptor (IL-12R) that is primarily expressed on activated T and NK cells. This high affinity IL-12R is composed of two subunits designated as Rb1 and Rb2. Mutations in the gene encoding the b1 subunit have recently been identified in seven patients having impaired, yet not abrogated, IFN γ secretion. The diagnosis of disseminated BCG infection was established in two Tunisian patients belonging to unrelated families. Patient 1 is a girl born to consanguineous parents, vaccinated with BCG at birth. Intrafamilial segregation of polymorphic microsatellites flanking the IL-12Rb1 gene revealed a large region of homozygosity, which is consistent with the inheritance of a common pathogenic IL-12Rb1 allele from the related parents. The sequencing of subcloned RT-PCR products have shown two different cDNAs, one with a deletion of 31 bp introducing a premature stop codon at nucleotide positions 605 to 607 (TGA) in the extracellular domain coding region. The other one has a deletion of 171 bp that conserves the reading frame. The analysis of IL-12Rb1 gene structure showed that these deletions correspond respectively to exon 6 and both exon 5 and 6 skipping. This is due to the (IVS5-2A>G) mutation. Patient 2 is a boy born to parents who were first cousins, he was vaccinated at one month of age. The sequencing of cloned PCR products of IL-12Rb1 cDNA reveals different mutated forms corresponding to incorrect splicing of intron 1 involving the use of cryptic splice sites. This rearrangement is due to the (IVS1+5G>A) mutation. At the genomic level, the parents are heterozygous for the mutation. Herein, we report new mutations in the IL12Rb1 gene that emphasize the essential role of IL-12 in the control of mycobacterial infections.

Molecular analysis of the deletion breakpoints of Parkin gene. *S. Asakawa*¹, *N. Hattori*², *A. Shintani*¹, *T. Sasaki*¹, *K. Kawasaki*¹, *A. Shimizu*¹, *T. Kitada*^{1,2}, *H. Matsumine*¹, *S. Minoshima*¹, *Y. Shimizu*³, *Y. Mizuno*², *N. Shimizu*¹. 1) Department of Molecular Biology, Keio University School of Medicine, Shinjuku-ku, Tokyo, Japan; 2) Department of Neurology, Juntendo University School of Medicine, Bunkyo-ku, Tokyo, Japan; 3) Department of Medical Genetics, School of Health Sciences, Kyorin University, Hachioji, Tokyo, Japan.

The Parkin was discovered as a pathogenic gene of the autosomal recessive juvenile parkinsonism (AR-JP). The Parkin gene consists of 12 exons with 1.4 kb of coding sequence and spans over 1.5 Mb on the q25.2-q27 region of human chromosome 6. A variety of mutations have been found in the Parkin gene of AR-JP patients of different ethnic origins. The majority (75%) of Japanese AR-JP patients had large exonic deletions which were more often in a region covering from exon 3 to exon 5. In order to analyze the molecular nature of the deletions, we started sequencing the entire Parkin gene in sharing the task with The Sanger Centre. Based on the sequences so far determined, we designed more than 147 pairs of PCR primer and generated a detailed deletion map using DNAs of one Israeli, one Taiwanese, one Korean, four Turkish, and 16 Japanese AR-JP families. The deletions were classified into 18 types. One type of exon 4 deletion was commonly found among 6 families. We determined the exact sequences of the breakpoints for 11 types of the deletion. Moreover, we were able to design FISH probes to detect each exon with flanking sequences which were successfully used for the diagnosis of compound heterozygotes. Furthermore, we also established the diagnosis of compound heterozygotes of exon deletions by quantitative PCR technique. The structure of Parkin gene and molecular natures of deletion breakpoints will be presented.

The spectrum of novel mutations in ATP2C1 in Hailey-Hailey Disease. *N. Brown¹, M. Tieu¹, J.J. DiGiovanna^{2,3}, S.J. Bale³, J. Uitto¹, G. Richard¹.* 1) Dermatology, Jefferson Medical College, Philadelphia, PA; 2) Dermatology, Brown University/Rhode Island Hospital, Providence, RI; 3) Genetics Study Section, NIAMS, NIH Bethesda, MD.

Hailey-Hailey disease (HHD) is an autosomal dominant skin disorder of impaired epidermal cell adhesion characterized by expanding erosions and crusted or fissured plaques in intertriginous areas, and by a late onset between the second and fourth decade. We have ascertained 3 unrelated individuals and 18 multiplex families with HHD of different origin, and confirmed and refined linkage of HHD to 3q24 in 10 multigenerational families. Recently, Hu et al. (Nature Genet. 24:61-65, 2000) cloned and identified the HHD gene ATP2C1, which encodes the high-affinity calcium pump hPMR1. ATP2C1 is highly expressed in human keratinocytes, and assumed to sequester excess cytosolic calcium into the Golgi. Mutations have been shown to perturb the intracellular calcium homeostasis of keratinocytes, and may thus confer a direct effect on the calcium-dependent assembly of desmosomes. Using CSGE followed by direct automated sequencing, we screened all 27 exons and flanking sequences of ATP2C1 in our cohort from the US, Germany and Italy. Thus far, we identified 7 distinct mutations in 8 families, all of which are novel. These include 1 nonsense and 3 missense mutations, 1 small deletion, and 2 splice site mutations. Mutation R806X was identified in 2 unrelated families of different origin, and is predicted to eliminate the last 3 exons of ATP2C1. In addition, 6 HHD families carried polymorphisms of the coding sequence, including 2 non-consequential SNP, a small deletion in the 3'UTR, and L62E. Our data confirm that most mutations of ATP2C1 are private and dispersed over different exons, although there might be a slight preponderance in the last third of the gene. Similar to the previous study and despite the convincing evidence for genetic homogeneity of HHD, disease-causing mutations in ATP2C1 were not detected in about 60% of all tested families. A systematic screening with allele-specific detection methods is underway to identify gross gene rearrangements, which may alter expression or function of hPMR1.

DHPLC as a Method for High Throughput, Cost Effective Mutation Screening: Application to Rett Syndrome.

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Rett syndrome is a neurodevelopmental disorder affecting females and is characterized by developmental regression, severe mental retardation and stereotypic hand movements. Recent studies have shown that 60-80% of girls with Rett syndrome have mutations within the X-linked gene encoding the methyl-CpG-binding protein 2 (MeCP2). We have received more than 270 samples for diagnostic mutation testing for Rett syndrome in a short period of time. Mutation screening was complicated by most cases representing de novo mutations and expression of the disease as a heterozygote. To efficiently screen this large number of samples, we have developed a "targeted sequencing" approach using a combination of denaturing high performance liquid chromatography (DHPLC), a mutation screening method that relies on heteroduplex detection, and direct sequencing.

We examined the sensitivity of DHPLC for detection of MeCP2 mutations by performing DHPLC analysis on 18 mutation positive samples, representing 10 different MeCP2 mutations, and 22 mutation negative samples as determined by direct sequencing. Heteroduplex peaks were detected for every mutation positive sample. These results indicated a high level of sensitivity, approaching 100%, for mutations within the MeCP2 gene. DHPLC has many advantages over conventional mutation screening methods, including semi-automated analysis of 96 PCR samples in less than 12 hours, the absence of fluorescent or radioactive labeling, and low relative cost per sample approximately \$0.40. Targeting samples for direct sequencing after DHPLC analysis proved to be a more efficient method for MeCP2 mutation detection by reducing the amount of sequencing performed on each sample and by localizing the mutation to a certain region of the MeCP2 gene.

Evidence of a correlation between germline mutations in the carboxy-terminus of tuberlin (*TSC2*) and pulmonary lymphangiomyomatosis. A. Astrinidis¹, G.D. Strizheva¹, T. Carsillo¹, W. Kruger¹, E.J. Sullivan², J.H. Ryu³, E.P. Henske¹. 1) Fox Chase Cancer Center, Philadelphia, PA; 2) US Food and Drug Administration, Rockville, MD; 3) Mayo Clinic, Rochester, MN.

Lymphangiomyomatosis (LAM) is an often-fatal lung disease that affects young women with tuberous sclerosis complex (TSC). The genetic and cellular mechanisms underlying TSC-associated LAM are unknown. LAM is characterized by a diffuse proliferation of abnormal smooth muscle cells in the lungs. Here we report the mutational analysis of 14 unrelated women with TSC-associated LAM. All 21 coding exons of *TSC1* and 41 exons of *TSC2* were analyzed for mutations using SSCP. We identified germline mutations in eight patients (one in *TSC1* and 7 in *TSC2*). Two patients had the same in-frame deletion in exon 40 and another patient had a missense mutation in exon 41. The mutation in exon 41 causes a change from arginine to cysteine in tuberlin (the *TSC2* gene product), that has not been previously reported in any TSC patient. Both the exon 40 and 41 changes occur in regions of tuberlin that are highly conserved between human, rat, mouse and pufferfish. We also detected loss of heterozygosity in the *TSC2* gene region in pulmonary LAM cells using laser capture microdissection. We conclude that (1) germline mutations in the extreme carboxy terminus of tuberlin can cause pulmonary LAM, indicating that this region of tuberlin may be important for controlling smooth muscle cell proliferation and (2) the two-hit tumor suppressor gene model applies to the pathogenesis of TSC-associated LAM. This is the largest series of women with LAM in whom mutations have been identified, and the first demonstration that the two-hit model applies to TSC-associated pulmonary LAM cells.

Pendred Syndrome, DFNB4 and PDS - Phenotype/Genotype Correlations. *C.A. Campbell, R.A. Cucci, S.D. Prasad, R.J.H. Smith.* Dept Otolaryngology, Univ Iowa, Iowa City, IA.

Pendred syndrome, an autosomal-recessive disorder characterized by congenital sensorineural hearing loss and goiter, is caused by mutations in PDS. The classical phenotype is one in which deafness is present at birth and thyromegaly, in the second decade. The hearing loss is associated with temporal bone abnormalities that range from isolated enlargement of the vestibular aqueduct (dilated vestibular aqueduct, DVA) to Mondini dysplasia. Mutations in PDS also cause DFNB4, a type of autosomal recessive non-syndromic hearing loss (ARNSHL). Whether specific PDS mutations are associated with a particular deafness phenotype is not known. The phenotypic differences observed between Pendred syndrome and DFNB4 may reflect the functional consequences of PDS allele variants, the genetic background on which these allele variants are found, or the effects of external factors.

To determine whether phenotypic and genotypic data can be correlated in persons with PDS mutations, we completed PDS mutation screening in persons with temporal bone abnormalities consistent with DVA or Mondini dysplasia. In persons in whom mutations were identified, we compared the PDS genotype to the clinical phenotype focussing on results of temporal bone computed tomography. To complete this comparative study, we also reviewed all other reports describing PDS disease-causing mutations. We found 15 different PDS allele variants in 34 persons. Of these mutations, 7 have been reported previously and 8 are new, bringing the number of PDS disease-causing mutations now known to 45. Our data suggest that a correlation may exist between clinical phenotype and PDS genotype.

Recent Developments in Diagnostic Testing for Hereditary Hemochromatosis. *M.T. Bashford, B.B. Roa.* Baylor DNA Diagnostic Laboratory, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Hereditary hemochromatosis is an autosomal recessive disorder of iron metabolism which is associated with mutations in the HFE gene (also known as HLA-H). The majority of patients are homozygous for the C282Y mutation and a minority are compound heterozygotes for the C282Y and H63D alleles. Our laboratory offers DNA testing for hereditary hemochromatosis as a clinical service. Since February of 1998, we have been testing for the C282Y and H63D mutations in HFE by an allele-specific oligonucleotide (ASO) hybridization assay. In August of 1999, Jeffrey, et al reported a single-nucleotide polymorphism (5569G/A) in intron 4 of the HFE gene. This polymorphism lies within the antisense primer-binding site commonly used for C282 mutation analysis, and can cause decreased amplification of the wild-type C282 allele. This can result in misdiagnosing a C282Y heterozygote as an apparent homozygous mutant. One study reported that the 5569G/A polymorphism was present in 15 of 31 (48%) of their apparent C282Y homozygous patients. However, subsequent studies suggest a lower frequency. Another published report found 8 out of 52 patients (15%) who were initially misdiagnosed as C282Y homozygotes due to this polymorphism. Our laboratory retested 121 patients who had been previously diagnosed as C282Y homozygotes. A redesigned primer pair corresponding to exon sequences was used for PCR and ASO analysis. We identified one misdiagnosed heterozygous patient (0.8%) in our cohort. Observed variation in frequencies may be due to population substructure or differences in clinical ascertainment between patient groups.

A second gene (TFR2) was recently associated with hemochromatosis in families of Sicilian origin. A single nonsense mutation, Y250X, was found in 6 patients from 2 families. We are currently testing anonymized DNA samples from patients previously referred for hemochromatosis testing to determine the frequency of the TFR2 Y250X mutation in this referral population. This will allow us to evaluate the frequency and utility of including the TFR2 Y250X mutation in our hereditary hemochromatosis panel.

Notch3 gene mutation analysis of individuals with possible CADASIL diagnosis. *G. Deng¹, M. Gaudette¹, T. Siddique^{1,2}*. 1) Neurology, Northwestern University Medical School, Chicago, IL; 2) Cell and Molecular Biology, Northwestern University Institute of Neuroscience, Chicago, IL.

CADASIL (Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is a rare adult onset syndrome most commonly characterized by stroke-like episodes, migraines with aura, mood disturbances, white-matter abnormalities on neuroimaging, and eventual dementia. While expression can vary greatly even among individuals of the same family, the age dependent penetrance of this disorder is thought to be complete no later than the sixth decade of life. Missense mutations leading to a loss or gain of a cysteine residue in the Notch3 gene, located on the long arm of chromosome 19, are thought to account for at least 90% of causative gene mutations in CADASIL patients. This study was conducted to further describe the clinical spectrum resulting from Notch 3 mutations. Therefore, DNA from twenty-five unrelated patients diagnosed with possible CADASIL was sequenced for exons 3, 4, 11, 18, and 19 of the Notch 3 gene. These five exons have been shown to harbor 90% of identifiable CADASIL mutations. SSCP analysis of the remaining 28 exons is ongoing. Four previously reported mutations were detected by sequencing; two exon 3 mutations (R90C) and two exon four mutations (R141C and R182C). Both variable intra- and interfamilial expression was evident. The low mutation detection rate of 16% (4/25) based on sequencing the 5 most common exons can be attributed to samples being analyzed on a possible rather than probable CADASIL basis. This criteria allowed for DNA sequencing on individuals with unexplained neurological disturbance and MRI visualization of cerebral ischemia, regardless of other symptoms or family history. Although, the entire gene analysis is not complete, the low detection rate may also imply that another locus or loci are responsible for CADASIL-like phenotypes.

FBN2 mutations in congenital contractural arachnodactyly (CCA). *S. Carmical, P. Gupta, D.M. Milewicz, E.A. Putnam.* Int Med/Med Gen, Univ Texas Med Sch, Houston, TX.

Congenital contractural arachnodactyly (CCA, or Beals syndrome) is an autosomal dominant disorder phenotypically similar to Marfan syndrome (MFS). CCA results from mutations in the FBN2 gene encoding fibrillin-2, an extracellular matrix protein found in 10-12 nm microfibrils. Thus far, all identified FBN2 mutations have clustered in exons 23 through 34, the region of the gene analogous to the location of mutations in FBN1 that produce the severe neonatal MFS phenotype. Using intron-based exon-specific primers, we amplified FBN2 exons 23 through 34 from CCA patient genomic DNA. SSCP analysis followed by sequencing, as well as direct sequencing, identified 4 new confirmed mutations in classic CCA patients. The first FBN2 mutation resulting in a premature termination codon (Y1421X) in exon 33 was identified. The other identified mutations include a third exon 26 splicing error, caused in this incidence by 3469+2, T->C. The missense mutations removing cysteines in EGF-like domains (C1239R in exon 28, and C1256W in exon 29) were analogous to those commonly identified in the FBN1 genes of MFS patients. In contrast, no mutations that alter amino acids important for calcium binding to EGF-like domains have been identified in FBN2, either reported here or in the 16 previously reported FBN2 mutations. Approximately 25% of the FBN1 mutations that disrupt EGF-like domains alter amino acids critical for calcium binding. Since calcium binding has been shown to be important for fibrillin stability, the lack of FBN2 mutations predicted to disrupt calcium binding to EGF-like domains may give insight into the molecular pathogenesis of FBN2 and FBN1 mutations.

Detection of point mutations in the dystrophin gene by genomic scanning with DOVAM-S. *C.H. Buzin¹, J. Feng¹, J. Yan¹, C. Serrano², D. Sangani², T.W. Prior², J.R. Mendell², S.S. Sommer¹.* 1) City of Hope National Medical Center, Duarte, CA; 2) Ohio State University Medical Center, Columbus, OH.

Duchenne muscular dystrophy (DMD) is caused by mutations in dystrophin, one of the largest known genes that spans nearly 2.4 Mb of genomic DNA, contains 79 exons, and encodes a 14 kb transcript. About 60% of the mutations in the gene involve large deletions or duplications. The remaining 40%, presumably single nucleotide substitutions or microdeletions/insertions, have been difficult to identify because of the size of the gene and the lack of known hotspots for point mutations. A recently developed method, Detection of Virtually All Mutations-SSCP (DOVAM-S), scans up to 500 kb of genomic DNA in five SSCP gels, each run under different electrophoresis conditions. In blinded analyses of the factor VIII and ATM genes, the method detected all of 180 mutations located in the regions scanned. In this study, a total of 2 Mb of genomic sequence, including all exons and most adjacent intronic splice regions of the dystrophin gene, was scanned by DOVAM-S in 93 DMD patients ascertained both clinically and by muscle biopsy. Prior screening excluded most patients with large deletion and duplication mutations. Point mutations occurred in 68 patients; exonic deletions occurred in five. Of 62 different point mutations, there were 25 microdeletions/insertions, 30 nonsense and 7 splice site mutations. Most mutations were detected in only a single patient, as expected for an X-linked lethal disease in which mutations have a half life of two generations. Mutations were detected in 73 of 93 patients (79%). The remaining mutations may be in additional regulatory regions or deep within introns. We speculate that the data in aggregate are most compatible with genetic heterogeneity within the DMD phenotype. The use of DOVAM-S to detect a majority of the point mutations, in addition to previous methods for the detection of large deletions and duplications, suggests that mutations can now be detected on a clinical time scale in over 90% of DMD patients.

Genetic heterogeneity and functional candidate gene analysis in Joubert syndrome. *I.P. Blair, R.R. Gibson, P.F. Chance.* Dept Pediatrics, Univ Washington, Seattle, WA.

Joubert syndrome (JS) is a rare autosomal recessive malformation syndrome involving agenesis or dysgenesis of the cerebellar vermis with accompanying brain stem malformations. The disease is characterized by hypotonia, developmental delay, abnormal respiratory patterns, and abnormal eye movements. The biochemical and molecular mechanisms underlying the disorder are unknown. The syndrome is clinically heterogeneous and this together with linkage analyses indicates that there is also genetic heterogeneity. A previous genome-wide linkage study in ten JS pedigrees did not identify a specific chromosomal locus for the disorder. More recently, homozygosity mapping identified a Joubert locus on chromosome 9q34 in a consanguineous family of Arabian/Iranian origin and excluded the same locus in a second pedigree (Saar et al. AJHG 1999). An attractive JS candidate gene, *BARHL1*, was recently localized to 9q34. We investigated the involvement of *BARHL1* in Joubert by detailed physical mapping in relation to the 9q34 Joubert locus. The gene was excluded when it was localized proximal to the candidate interval. We have also undertaken haplotype analyses in 26 Joubert pedigrees including several consanguineous families, using the markers that define the locus on chromosome 9q34. We have found no evidence of homozygosity in these pedigrees that would indicate association with the 9q34 locus, strongly suggesting that one or more major loci for Joubert syndrome are yet to be identified. We are therefore selecting functional candidate genes from throughout the genome for mutation analysis in our cohort of Joubert families. Mutation analysis is being carried out using a combination of dideoxy fingerprinting and direct sequencing. To date, no mutations have been identified in candidate genes *FGF8* and *ENG1* & 2 although more families remain to be investigated. Similarly other strong candidates await investigation including *GBX2*, *MATH1*, *PAX2*, and *PAX5*.

Prevalence of Norrie Disease Gene Mutations in Patients with Advanced Retinopathy of Prematurity. M.

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Retinopathy of prematurity (ROP) is a multifactorial disorder which causes fibrovascular tissue changes that affect the retina of low birth-weight and short gestational age infants. It is a leading cause of blindness in premature children. To determine the prevalence of Norrie disease (ND) gene mutations in patients with advanced ROP, we have undertaken a large scale analysis of leukocyte DNA. The patients set contained 100 pre-term babies including 3 pairs of dizygotic twins, 2 pairs of monozygotic twins and 6 patients with stage 3 ROP that had regressed without treatment. The birth weight and gestational age ranged from 600-1300g (mean = 882g) and 20-30 weeks (mean = 26 weeks) respectively. This group of patients contained 63% males and 37% females (98% Caucasians and 2% African-Americans). A group of 130 unrelated, randomly selected healthy adults served as control subjects. The leukocyte DNA was extracted, amplified by the polymerase chain reaction (PCR) and analyzed by single strand conformation polymorphism (SSCP), G/T and A/C scanning as well as by DNA sequencing. All three exons including the splice sites and the 3'-untranslated region were screened. Of the 100 patients analyzed, two patients (with advanced ROP) showed a mobility shift in the DNA. In one patient this mobility shift was caused by a 12 bp insertion in exon 1 and in the second, a 14 bp deletion in the same exon as evidenced by direct sequencing of the amplified products. Similar analyses of exons 2,3 and 3'-untranslated region failed to detect additional mutations in the gene. None of the 130 unrelated normal controls revealed similar changes. Since exon 1 of the ND gene has a role in controlling the expression of the gene, it is possible that the insertion and deletion mutations identified in the patients have some relationship to advanced ROP. Such mutations may affect message stability and/or translatability of mRNA. Taking into account the above results as well as other studies, it appears that ND gene mutations can account for 5% cases of advanced ROP.

Direct sequencing of dystrophin gene. *R.R. Bennett, L. Kunkel.* Genetics, Children's Hospital, Boston, MA.

The price of sequencing fragments up to 600 bases in one direction has reduced over the last few years to between \$5.00 and \$7.00 per fragment. At this price, direct sequencing is the only and best alternative for detecting point and other mutations in any gene including one as large as the dystrophin gene. The author has designed and will present a set of primers which will amplify and sequence all 79 exons plus 5 prime (3 primer pairs) and 3 prime (7 primer pairs) UTR's. Each fragment includes the exon's entire coding region plus at least 50 bases of intronic sequence on each side of the exon. The author has chosen 7 boys with confirmed DMD and no major deletion. Having sequenced only 20 exons and the 5 prime UTR as of June 7, 2000, stop mutations in two of the boys and possible phenotype causing missense mutations in two others have been found. All seven boys mutations should be found and all 79 exons plus UTR's sequenced by the time of the meeting.

Nonhomologous recombination leading to duplication of the PLP gene in Pelizaeus-Merzbacher disease: analysis of the DNA rearrangement breakpoints. A. Iwaki¹, M. Ototsuji¹, K. Kurosawa¹, G.R. Howell², Y. Fukumaki¹. 1) Inst. Genet. Information, Kyushu University, Fukuoka, Japan; 2) The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, UK.

Complete duplication of the proteolipid protein (PLP) gene is the most common cause of Pelizaeus-Merzbacher disease (PMD), an X-linked disorder characterized by dysmyelination in the central nervous system. We identified seven Japanese PMD families with the PLP gene duplication by quantitative multiplex PCR analysis. The duplications in six families were confirmed by interphase fluorescence in situ hybridization (FISH) using a PAC clone containing the PLP gene. Furthermore, the extent of the duplicated region was systematically examined using 15 PAC probes along the Xq22 region by interphase FISH. Size of duplications varied between the families from 0.3 to 1 Mb, and positions of both centromeric and telomeric breakpoints were variable. We found one family, in which duplication was apparent by quantitative PCR analysis but not by interphase FISH, suggesting the size of duplication to be relatively small. To elucidate the molecular mechanism of the genomic rearrangement in this family, we determined the duplicated region by quantitative multiplex PCR with 16 pairs of STS primers around the PLP gene. Field-inversion gel electrophoresis and Southern hybridization identified rearranged junction fragments. Sequencing of the endpoint and junction fragments amplified by PCR revealed that there is no homologous sequence except 3 nucleotides between the centromeric and telomeric ends, indicating nonhomologous recombination resulted in the tandem duplication of 285 kb. This finding together with the size variation between PMD families suggested instability of genomic structure of the Xq22 region leading to the PLP gene duplication.

Molecular characterization of minor gene rearrangements in Korean patients with familial

hypercholesterolemia. *S.H. Kim¹, U.K. Kim¹, J.J. Chae¹, Y. Namkoong², M.Y. Oh³, C.C. Lee¹.* 1) School of Biological Sciences, Seoul National Univ, Seoul, Korea; 2) Department of Biology, Kangung National Univ., Kangung 210-702, Korea; 3) Department of Biology, Cheju National Univ., Cheju 690-756, Korea.

Familial hypercholesterolemia (FH) is an autosomal dominant disorder caused by mutations in the low-density lipoprotein (LDL) receptor gene. To assess the frequency and nature of mutations of the LDL receptor gene in Korean FH patients, we screened 104 unrelated FH heterozygotes by single strand conformation polymorphism (SSCP) analysis. All of the 18 exons and a promoter region were amplified and analyzed. Fifteen different LDL receptor defective alleles were identified, comprising eleven missense mutations, two nonsense mutations, one splicing mutations and one small deletion mutations. Although more than 300 different mutations of the LDL receptor gene have been reported so far, nine of the mutations described in this study were novel ones, that have not been detected anywhere. Totally, fifteen different mutations were found in 33 unrelated FH patients and this implies that the LDL receptor gene mutations account for 32 % (33/104) of the FH causing genes in Korean population. Of the fifteen different mutations detected in the present study, the missense mutation, Pro664 Leu in exon 14 was detected in DNA samples from ten unrelated FH probands. Thus, the Pro664 Leu mutation is a relatively common that account for ~10% of the FH-causing genes and for ~30% of the LDL receptor gene mutation in the Koreans and is regarded as a founder-mutation, though the frequency is not high enough to that of the founder-mutations detected in other populations. Relatively various mutations are detected in Korean FH patients and any common mutation which represent founder effect was not detected. Nine mutations of fifteen mutations are Korean specific mutations which have not been reported anywhere. These results suggest that the molecular genetic background of the LDL receptor gene mutations causing FH in the Koreans is different from Western society.

***JAGGED1* mutations in Alagille syndrome: Update and Inheritance.** M. Meunier-Rotival¹, R.P. Colliton², C. Crosnier¹, L. Bason², C. Driancourt¹, I.D. Krantz², M. Hadchouel¹, N.B. Spinner². 1) INSERM U347, Kremlin-Bicetre, France; 2) Children's Hosp Philadelphia, Div Human Gen & Mol Biol, Philadelphia, PA.

Heterozygous mutations in the *JAGGED1* gene cause Alagille syndrome (AGS, MIM118450), an autosomal dominant disorder characterized by developmental abnormalities affecting the liver, heart, vertebrae, eyes, and face. The experience from our laboratories, in combination with the published literature has shown that mutations can be detected in 60-70% of patients. Here we present all currently available data, identifying 212 mutations from laboratories around the world (92 from France, 14 from Italia, 13 from Japan, 93 from the United States). Of these 212 mutations, 121 are frameshifts (57%), and 91 are point mutations (43%). Hot spots of mutations are observed in the gene. Because there are 163 different kinds of mutations and because the most frequent one accounts for only 12 of the 212, it will be difficult to develop a straightforward molecular screening test. *JAGGED1* encodes a transmembrane protein which is a ligand of Notch receptors. All mutations map in the part of the gene encoding the extracellular and transmembrane domains of the protein (including 4 in the signal peptide, 8 in the Delta-Serrate-Lag2 domain, 121 in the EGF repeats, 28 in the cysteine-rich region, and 3 in the transmembrane domain). The majority of mutations (151/212 or 71%) could lead to truncated unanchored proteins. Another 2 (1%) lead to shortened proteins and 29 (14%) result from splice defects. There are 30 missense mutations (14%), most of them at amino-acids conserved among species. Eight of the 9 missense mutations in EGF repeats lead to loss or gain of cysteine residues, as has been reported for NOTCH3 in CADASIL. There is no clear difference in the mutations found in series of patients from different countries. Inheritance of the mutations was reported in 125 cases: 79 were de novo (63%) and 46 (37%) were transmitted, 24 from the father and 22 from the mother. The Alagille syndrome phenotype shows variable expressivity. The same mutation of *JAGGED1* gives a range of expression, both within families and in unrelated patients. No genotype phenotype correlation can be made. meunier@infobiogen.fr.

Direct evidence of somatic mosaicism in tuberous sclerosis. *K. Mayer, S. Schirdewahn, H.-D. Rott.* Institute of Human Genetics, Erlangen, Germany.

Mosaicism occurs in all genetic disorders susceptible to spontaneous mutations and has consequences for:-the assessment of localized expression of multisystem disorders,-genetic counseling,-and molecular diagnostic testing. In tuberous sclerosis complex (TSC) two-thirds of cases are sporadic without of a family history usually caused by new mutations. First indications for mosaicism came from the observation of more than one affected child from parents without clinical signs of TSC. Haplotype analyses confirmed parental germ-line mosaicism. Parental somatic mosaicism is usually detected during mutation screening in the family of an index patient by a reduced ratio of the mutant-to-normal allele. Subsequent clinical investigation of the mosaic parents often fulfilled diagnostic criteria. In our series of 29 sporadic patients with clinical signs of TSC with an identified TSC1 or TSC2 mutation we directly discovered two cases of somatic mosaicism. One TSC1 splice site and one TSC2 nonsense mutation were initially detected through the sensitivity of the protein truncation test (PTT). Failure to confirm the mutations by sequence analysis of peripheral blood DNA suggested low level mosaicism. Subsequent demonstration of the TSC1 mutation in one of 20 individual PCR clones obtained from lymphocyte DNA and selective amplification of the mutated allele after enzymatic digestion of the wildtype allele in lymphocytes and buccal mucosa of the TSC2 patient proved low level mosaicism. Interestingly, the low proportion of mutated alleles were sufficient to be interpreted as heteroduplexes by DHPLC analysis. In both patients the diagnosis TSC had not been considered with the first clinical signs but during the course of later complaints which requested a TSC mutational screening: in the TSC2 mosaic patient an urogenital infection uncovered several renal angiomyolipomas; in the TSC1 mosaic patient a single sclerotic tuber interpreted as a hemosiderin deposit after an old cerebral bleeding took monosymptomatic TSC into account. Our two case reports illustrate the importance of considering somatic mosaicism in sporadic TSC cases with clinical symptoms restricted to single organs and the necessity of sensitive methods for their detection.

Mutation Analysis of EYA1 Gene Associated with Branchio-Oto-Renal Syndrome and Refining the BOR2

Region on Chromosome 1q. *S. Kumar*¹, *H.A.M. Marres*², *C.W.R.J Cremers*², *W.J. Kimberling*¹. 1) Dept Genetics, Boys Town Natl Research Hosp, Omaha, NE; 2) Department of Otolaryngology, University Hospital Nijmegen, The Netherlands.

Branchio-oto renal syndrome (BOR) is a human developmental disorder that includes external, middle and inner ear malformations, branchial cleft sinuses, cervical fistulas, mixed hearing loss and renal anomalies. It is inherited as an autosomal dominant pattern and affect at least 2% of profoundly deaf children and have estimated prevalence of 1 in 40,000. The phenotypic expression of the branchial arch as well as audiologic and renal development can be quite variable, even within the same family. The BOR gene has been localized to chromosome 8q13 and has been shown to be caused by mutations in the EYA1 gene. We have performed mutation analysis on more than fifty BOR families by heteroduplex followed by sequence analysis of sixteen EYA1 exons. To date, using this approach, we have identified thirteen novel mutations. More than 65% of our BOR families have not shown mutations in the EYA1 gene. It is not clear whether the inability to detect the mutation is due to locus heterogeneity or because the mutation lies in the non-coding region of the EYA1 gene. Therefore, we performed genetic linkage analysis on multigenerational BOR type families with 8q markers. Our current results indicate that three large BOR type families did not show linkage with 8q markers suggesting the involvement of more than one gene. A genome-wide search is performed and identified the second genetic locus involved in branchiogenic disorder on chromosome 1q. The localization region is narrowed from 22 cM to 9 cM genetic interval between markers D1S422 and D1S2622. The present results, together with mutation screening and genetic linkage study, demonstrate genetic heterogeneity. These results provide the basis for a molecular-genetic testing that will help the clinical evaluation and genetic counseling of members of BOR families. Further characterization of EYA1 mutation and identification of other BOR genes will significantly help in defining the spectrum of defects associated with branchial and hearing anomalies.

Molecular determination of somatic mosaicism in typical NF2 patients. *L. Kluwe¹, V. Mautner², W. Xin³, L.B. Jacoby³, C.J. Willett³, M. MacCollin³.* 1) Neurology, University Hospital, Hamburg, Germany; 2) General Hospital Ochsenzoll, Hamburg, Germany; 3) Neurology and Neurosurgery, Massachusetts General Hospital, Charlestown, MA.

In our previous studies, we have shown the importance of somatic mosaicism in the generation of variant NF2 phenotypes such as unilateral vestibular schwannoma in the presence of other NF2 related tumors. The purpose of the current study was to examine the extent to which mosaicism is present in persons with typical NF2 (bilateral vestibular schwannomas). As part of our ongoing work on the molecular genetic basis of NF2, we studied tumor specimens from a total of 26 founders (persons with bilateral vestibular schwannomas with unaffected parents) in whom no germline *NF2* change was found by exon scanning. Exon scanning and directed sequencing was used to define *NF2* gene mutations in these tumors. Microsatellite analysis of flanking and intra genic markers in blood-tumor pairs was used to detect hemizygosity as a presumed "second hit." Typical truncating mutations were detected in 14 of 26 tumors and a missense generating change of unclear significance was detected in a single tumor. In 6 individuals, mosaicism was confirmed by the presence of identical mutations in pathologically and/or anatomically distinct second tumors. In 6 individuals, mosaicism was highly suspected because of the presence of mutation and loss of heterozygosity, but no second tumor was available for confirmation. Of the 12 mutations associated with definite or probable mosaicism, all but one would be predicted to produce gross truncation through nonsense or frameshift. 10 of the 12 involved C to T transitions at CpG islands.

Mosaicism may account for a significant percentage of founders with typical NF2, and mosaic individuals may not manifest changes in blood samples on exon scanning. Molecular genetic analysis of the *NF2* gene should begin in tumor tissue whenever possible, especially when examining a founder. Further work is needed to determine the range of phenotypes associated with mosaicism at this locus.

Analysis of Mutations in the Sarcoglycan Genes in Patients with Limb-Girdle Muscular Dystrophy. V.

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Limb-girdle muscular dystrophy constitutes a genetically heterogeneous group of disorders including the sarcoglycanopathies which are caused by mutations in the genes encoding alpha, beta, delta, and gamma sarcoglycan. We have developed an assay for mutation screening in the sarcoglycan genes, consisting of multiplex PCR, CSGE, and DNA sequence analysis. To date, clinical samples from 37 patients with known or suspected sarcoglycanopathies have been tested in our laboratory and a molecular diagnosis was obtained in 34 patients. Of the mutations identified, 13 are new mutations and include mostly frame shift and splice site mutations. In three sarcoglycanopathy patients in which only one mutation was identified by our standard assay, additional tests including Southern blot, long-range PCR and DNA sequence analysis have been performed to identify the second mutation. A 169bp deletion in the promoter region of the alpha sarcoglycan gene has been identified in a patient heterozygous for an alpha sarcoglycan point mutation. In two other related patients heterozygous for a point mutation in the beta sarcoglycan gene, preliminary results indicate a rearrangement that includes exon 2. While macrodeletions have been reported in gamma sarcoglycan gene, this is the first time that a large deletion or rearrangement has been described in the alpha and beta sarcoglycan genes, respectively. In conclusion, we have developed an assay for mutation screening in the four sarcoglycan genes and identified mutations in 92% of patients with known or suspected sarcoglycanopathies. Our results suggest that additional analyses may be needed to achieve a molecular diagnosis in some patients, since unsuspected macrodeletions or rearrangements may be present in the sarcoglycan genes.

Detection and Functional Analysis of Mutations in *Jagged1*. *M.L Heritage*¹, *J.C MacMillan*², *G.J Anderson*¹. 1) Clinical Sciences Unit, Queensland Institute of Medical Research, Brisbane, Queensland, Australia; 2) The Queensland Clinical Genetics Service, Royal Brisbane Hospital, Brisbane, Queensland, Australia.

Alagille syndrome (AGS) is an autosomal dominant disorder resulting from mutations in the human *Jagged1* gene and is characterized by abnormal development of the liver, heart, skeleton, eye and face. *Jagged1* encodes a ligand for the Notch receptors, which mediate cell-cell interactions during development. The aims of this study were to identify *Jagged1* mutations in an Australian AGS population and to determine the functional significance of these mutations. *Jagged1* mutations were detected by SSCP and confirmed by sequencing. Splice-site mutations were analysed by RT-PCR using patient RNA. To examine the effect of missense mutations on *Jagged1* function, a full-length wild-type clone was generated by RT-PCR and a C-terminal FLAG epitope tag was added. Mutations were introduced using site-directed mutagenesis. COS-7 and HeLa cells were transfected with these constructs and analysed by immunofluorescence microscopy and western blotting using a FLAG monoclonal antibody. We identified 12 *Jagged1* mutations in 15 (68.2%) of the 22 AGS cases recruited for this study. The mutations include 3 small deletions (25%), 2 small insertions (16.6%), 3 missense mutations (25%), 2 nonsense mutations (16.6%) and 2 splice site mutations (16.6%). One of the splice-site mutations was analysed further and abnormal splicing of exon 11 with consequent premature termination of translation in exon 12 was observed. The effects of the missense mutations R184C, C229Y and G386R on the subcellular localisation of *Jagged1* was studied in transfected COS-7 and HeLa cells. Wild-type *Jagged1* was expressed on the plasma membrane and also at various intracellular sites. None of the missense mutations studied had any effect on the expression of *Jagged1* on the cell surface, and the staining patterns observed were similar to those of wild-type protein. Since these missense mutations do not appear to influence the cell surface presentation of *Jagged1*, it is most likely that the phenotypic consequences of the mutations result from defects in the interaction of the mutant proteins with their Notch receptor targets.

Deletion mutations in the survival motor neuron and neuronal apoptosis inhibitory protein genes in 74 spinal muscular atrophy patients. *R. Majumdar¹, M. Al Jumah¹, S. Al Rajeh^{1,2}, E. Chaves-Carballo³, A. Awada¹, S. Al Shahwan⁴, M. Salih⁵, S. Uthaim¹.* 1) Neurogenetics Laboratory, Dept. of Medicine, King Fahad Natl Guard Hospital, Riyadh, Saudi Arabia; 2) Div. of Neurology, King Saud University, Riyadh, Saudi Arabia; 3) Dept. of Neursciences, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia; 4) Dept. of Pediatrics, Military Hospital, Riyadh, Saudi Arabia; 5) Dept. of Pediatrics, King Saud University, Saudi Arabia.

Spinal muscular atrophy (SMA) is an autosomal recessive disorder occurring at a rate of 1 in 10000 birhts. The phenotype results from death of the anterior horn cells of the spinal cord, resulting in symmetrical muscle weakness and wasting. Deletions or mutations in two genes, telomeric form of survival motor neuron (SMNtel) and the neuronal apoptosis inhibitory protein (NAIP), are known to be associated with SMA. The prevalence of SMA cases in Saudi Arabia is much higher than the European and North American population. In this study we included 74 Saudi SMA patients [SMA type I (32 patients), SMA type II (21 patients) and SMA type III (21 patients)] following SMA diagnostic criteria. We examined the deletion or interruption of the SMN and NAIP genes by using polymerase chain reaction (PCR) followed by restriction site assay. The homozygous deletions of exons 7 and 8 of the SMNtel gene were detected in 95% and 85%, respectively, of all SMA patients. The homozygous deletion of exon 5 of the NAIP gene (NAIPex5) was detected in 77% of all patients. Our results demonstrate that both the SMNtel gene deletion and the SMN gene conversion events occur in Saudi SMA patients, as expected. The deletion rate of NAIPex5 in Saudi SMA patients is the highest compared with SMA patients of other ethnic groups. Significantly, the deletions of both the SMNtel and NAIP genes are more common in Saudi SMA patients, irrespective of types, compared with SMA patients of other ethnic groups.

Novel and Recurrent Mutations in Laminin 5 Subunit Genes (LAMA3 and LAMB3) in Junctional Epidermolysis Bullosa: Phenotype/Genotype Correlations. *A. Nakano¹, S.C Chao¹, E. Pfendner¹, L. Pulkkinen¹, D. Murrell², V. Sybert³, J. Uitto¹.* 1) Dermatology, Jefferson Medical College, Philadelphia, PA; 2) University of New South Wales Sydney, Australia; 3) University of Washington Seattle, WA.

Junctional epidermolysis bullosa (JEB) is an autosomal recessive blistering disease, which is divided to two major subtypes, Herlitz (lethal; H-JEB) and non-Herlitz (non-lethal; nH-JEB) variants. Laminin 5, the candidate protein/gene system at fault in JEB, consists of three subunit polypeptides, the alpha 3, beta 3 and gamma 2 chains, and the corresponding genes, LAMA3, LAMB3 and LAMC2, are shown to harbor mutations in JEB. In this study, we have examined a cohort of 16 families with nH-JEB, and H-JEB for mutations in the laminin 5 genes. The mutation detection strategy consisted of assay for the two recurrent hotspot mutations R635X and R42X in LAMB3, followed by heteroduplex scanning by conformation-sensitive gel electrophoresis of LAMB3, LAMC2 and LAMA3, and automated nucleotide sequencing. The results revealed the presence of 22 distinct mutations in the two genes (LAMA3 and LAMB3), 16 of them being novel, nine of them residing in LAMA3. Among LAMB3 mutations, the hotspot mutations R635X and R42X were found in seven and one alleles, respectively. All H-JEB cases were homozygous or compound heterozygous for PTC-mutations predicting absence of laminin 5, in part due to accelerated mRNA decay. Surprisingly, one nH-JEB case harbored similar combination of mutations in LAMB3. In three other nH-JEB cases mutations consisted of PTC-mutations combined with missense or splicing mutations in LAMB3, or a missense mutation combined with a splicing mutation in LAMA3. These data support the following conclusions on the phenotype/genotype correlations in JEB: PTC-mutations in both alleles of any of the three genes encoding laminin 5 polypeptides are associated with H-JEB, while majority of the nH-JEB cases harbor a missense mutation or in-frame deletion at least in one allele.

Breakpoint characterization of tuberous sclerosis TSC1 and TSC2 gene deletions. *L. Longa, A. Brusco, A. Saluto, S. Polidoro, S. Padovan, A. Allavena, N. Migone.* Dept Genetics, Biology & Biochem, Univ Torino, Torino, Italy.

We screened 192 Italian TSC patients for TSC1 and TSC2 gene deletions through Southern blot of EcoRI+HindIII double-digested DNA. Three TSC1 deletions ranging in size from 1 to 7.6 kb, and thirteen TSC2 deletions from 1 to more than 260 kb were found. All TSC1 deletions were located in the 3' end of the gene: two involved exons 21-22, and one exons 21-23. TSC2 deletions were more heterogeneous, spanning internal exons only (4 cases), the first half of the gene including 5'UT and upstream regions (3), the TSC2 tail and PKD1 segments (2), or completely eliminating TSC2 plus a portion or the entire PKD1 (4). We sequenced the breakpoints of all TSC1 deletions and of 4 internal TSC2 deletions from long range PCR products. The mechanism of recombination appeared to be Alu mediated in 3 of 4 TSC2 deletions: an AluSg in IVS2 recombined with a AluSq in IVS9, while in IVS14/IVS21 and in IVS15/IVS29 breakpoints two AluY elements were involved. The breakpoint in the fourth TSC2 deletion occurred between IVS15 and IVS16, within a CAAGGG tract embedded in a region of no apparent homology. The 5'-breakpoints of all three TSC1 deletions were located in IVS20. Interestingly, two mapped in a 45-bp interval; the third one occurred 2.3 kb downstream. In none of them a homology region could be found within the breakpoint, except for an AG in one patient and a GAT in another. The third deletion breakpoint was centered in an 8-bp symmetric element (TGTT/TTGT). Short sequence motifs with an axis of internal symmetry are often observed at or near small deletions in humans (80% of 1828 deletions in HGMD). Finally, a polypurine rich region was observed 40-80 bp upstream or overlapping the breakpoint in 1 and 2 TSC1 deletions, respectively. In conclusion, the preferential involvement of IVS20 should help to set up efficient screening tests for TSC1 deletions in diagnostic settings. Work supported by Telethon, Italy (project E.730) and by "Assoc. E. & E. Rulfo per la Genetica Medica". Anna Allavena is recipient of a fellowship from "Associazione Italiana Sclerosi Tuberosa".

Rett syndrome: analysis of MECP2 mutations in Brazilian patients. *F.T. Lima¹, L.R. Vasques², S.M.N. Garcia², F. Kok³, P.G. Otto², L.V. Pereira².* 1) Centro de Genetica Medica, EPM-UNIFESP, SP, Brazil; 2) Dept. Biologia-IB, Universidade de Sao Paulo, SP, Brazil; 3) Dept. Neurologia-FM, Universidade de sao Paulo, Sp, Brazil.

Rett syndrome (RS) is an X-linked dominant progressive neurodegenerative disorder, with an estimated prevalence of one in 10,000-15,000 female births. After a period of normal development, patients develop mental retardation, loss of expressive speech, acquired skills and purposeful use of hands. Mutations in the methyl-CpG binding protein (MECP2) gene were recently found in RS patients. This gene is located in Xq28 and codes for a protein involved in transcription repression and gene silencing. Mutations in the MECP2 gene are found in 20-80% of the RS patients, depending on the population studied. In this work, mutation screening in the MECP2 gene was performed in 20 sporadic Brazilian RS patients and a pair of monozygotic twins. Sequencing of the coding region and splice sites revealed already described mutations in 43% of the patients. Mutation T158M, in the MBD region of the protein, was present in 4 patients. Two patients and the pair of twins had the R168X mutation, whereas one patient presented the R270X mutation in the TRD region of the protein. No mutations were detected in any of the parents available for investigation. These results confirm the existence of a mutational hot-spot in CpG dinucleotides in the MBD and TRD coding regions of the MECP2 gene associated with RS. Mutations in non-coding regions or the existence of a putative second X-linked RS gene could be explanations for the remaining cases of RS where no MECP2 mutations were found. FUNDING: FAPESP.

Lowe syndrome carrier testing by denaturing HPLC. *T. Lin, S.F. Suchy, R.L. Nussbaum.* Genetics Dis Res Branch, NHGRI/NIH, Bethesda, MD.

The oculocerebrorenal syndrome of Lowe (OCRL) is an X-linked genetic disorder involving the eyes, kidney, and nervous system. The OCRL1 gene encodes ocr1, a 105 kD phosphatidylinositol (4,5) biphosphate [PtdIns (4,5)P₂] 5-phosphatase that is deficient in OCRL patients. The diagnosis can be confirmed in affected males by enzyme assay, however the biochemical assay cannot be used for carrier detection. While a slit-lamp examination of the ocular lens has been shown to be a highly sensitive (>97%) method for detecting carriers; it requires an experienced ophthalmologist. A more consistent method for carrier detection is essential. Because most families have unique mutations, carrier diagnosis by molecular analysis is limited to families in which the mutation is known or in which linkage is informative. Thus a specific DNA analysis must be tailored for each family. Denaturing HPLC (DHPLC) is a heteroduplexes based, highly sensitive (92-96%) method for detecting unknown mutations. DHPLC is reportedly more sensitive than SSCP, DGGE and PTT and is less expensive than direct sequencing. Among the 73 reported mutations in the OCRL1 gene (23 exons), 93% of the mutations are located in exons 10-23 and 20.5% are in exon 15. We have designed new PCR primers for each exon from 10 to 23. The resulting amplicons are 173 to 807 bp. Our protocol for identification of unknown mutations by DHPLC is to screen exon 15 followed by the remaining exons 10 to 23. The mutated exon will then be sequenced. We have identified two new mutations by this method. One is a single nucleotide insertion (T) between 2498-2499 in exon 22 that causes truncation of the protein and a loss of PtdIns (4,5)P₂ 5-phosphatase activity. A second mutation, in exon 15, was identified as a 1547G>T missense mutation that causes a loss of enzyme activity.

Report on mutations characterized in the choroideremia gene. *K.E. McTaggart, M. Tran, D.Y. Mah, P. Haney, I.M. MacDonald.* Ophthalmology, Univ Alberta, Edmonton, AB., Canada.

Choroideremia (CHM) is an X-linked disorder characterized by progressive degeneration of the choroid, retinal pigment epithelium (RPE), and retina. The choroideremia gene encodes Rab Escort Protein-1 (REP-1). All reported mutations in the CHM gene result in either the truncation or absence of the normal gene product. The clinical diagnosis of choroideremia may thus be confirmed by the absence of REP-1 through Western analysis using the REP-1 antibody, 2F1. Mutation analysis was undertaken in 43 CHM families by SSCP-PCR and direct sequencing. Mutations were characterized in 40 families. Two families had deletions of the entire CHM gene and two had deletions of exon 1 and greater than 57kb of upstream sequence. 27 different mutations were characterized (partial deletions, insertions, and point mutations). Of these, 15 have not previously been reported by our group or other groups. Different families with the same mutation are not related. For example, one of the mutations in exon 6, is present in six of our families and is reported in several other publications. Mutation analysis did not detect a mutation in three families, although the absence of REP-1 by Western analysis confirmed the clinical diagnosis. The presence of a mutation cannot be ruled out in these cases however, because our mutation screen detects mutations only in exons and consensus splice sites. Mutations could thus exist within the promoter or could create alternate splice sites within introns. Our studies confirm that all mutations in CHM result in either the truncation or absence of the CHM gene product.

NEW MUTATIONS IN MYOSIN VIIA GENE IN THE PORTUGUESE POPULATION. *A. Mena¹, J. Camara¹, H. Vieira¹, M. Vitorino¹, H. Caria¹, M. Simao², I. Galhardo², T. Netta², O. Dias², M. Andrea², C. Correia¹, G. Fialho¹.*
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Allelic mutations in myosin VIIa gene (MYO7A) have been shown to result in syndromic (Usher syndrome - USH1B) and in nonsyndromic dominant (DFNA11) and recessive (DFNB2) forms of deafness. In the present study, screening of mutations in MYO7A gene has been carried out, by SSCP, in a sample of 130 unrelated, normal hearing individuals from the Portuguese population, in order to determine the prevalence of mutations in this gene. Families affected with nonsyndromic recessive deafness (NSRD) and negative for mutations in the coding region of GJB2 gene were also analysed. This report presents the results obtained so far for exons 4 and 7. Analysis of exon 4 revealed that 27% of the Portuguese population presents the IVS3nt88C-T polymorphism, already described by Weston et al. (1996). Regarding exon 7, a novel mutation Ala226Thr was found in one heterozygotic individual. Its functional significance needs to be elucidated, eventually confirming the hypothesis that a substitution of the hydrophobic alanine for a polar threonine may affect the charge of the surrounding motor region, where this mutation is localized, and consequently its function. As regards the analysis of the affected families, a new polymorphism IVS6nt40C-T was detected. Additional studies on these mutations are presently being performed.

Mutations within the IC78 (DNAI1) gene in patients with Primary Ciliary Dyskinesia (PCD). *M. Zariwala, P.G. Noone, A. Sannuti, S. Minnix, L.E. Ostrowski, Z. Zhou, J.L. Carson, M.R. Knowles.* Pulmonary / CF Research and Treatment Center, University of North Carolina at Chapel Hill, NC.

Primary Ciliary Dyskinesia (PCD) is a genetically heterogeneous disorder, caused by abnormal ciliary structure and function, and characterized by defective mucociliary clearance and bronchiectasis. PCD is usually inherited as an autosomal recessive trait, but its genetic origin has remained elusive. PCD patients (n=24) and parents (n=27) in 14 families were clinically phenotyped, including detailed analyses of nasal ciliary ultrastructure. Seven unrelated families with clear-cut defects in outer dynein arms (ODAs)(i.e. absent or near-absent ODAs) were selected using intragenic polymorphisms to test the possible involvement of the recently cloned IC78 (DNAI1) gene, which is an intermediate chain dynein belonging to the family of WD-repeat proteins.

We detected a previously reported mutation (Pennarun et al; Am. J. Hum. Genet. 65:1508-1519, 1999), an insertion of T at splice donor site in the +3 position of intron 1 in two families; one with total absence and one with near-complete absence of ODAs. It was inherited from the mother in both families and not present in the unaffected siblings. Complete genomic sequencing of the IC78 gene yielded codon 568 of exon 17 as another mutational site in both families. One family had a missense mutation G1874C (W568S) in an affected individual (inherited from father); the unaffected sibling was a carrier. The other family had a nonsense mutation G1875A (W568X) detected in an affected individual (inherited from father) which was not present in the unaffected sibling. The tryptophan at position 568 is a highly conserved residue in the WD-repeat region, and a mutation would be predicted to lead to abnormal folding of the protein. In conclusion, we detected two potential mutational hotspots in IC78 gene; one in intron 1, and the other in codon 568 in two PCD families. (GCRC #00046).

Allelic heterogeneity of autosomal recessive juvenile parkinsonism in an internal isolate from Colombia. *N.*

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We report the molecular characterization of three multiplex families from the province of Antioquia (Colombia) with Autosomal Recessive Juvenile Parkinsonism. Linkage an haplotype analysis using markers in 6q25.2-27 indicated that parkinsonism in these pedigrees is linked to the parkin gene (maximun lod score of 3.03) but that they carry two different mutant haplotypes. Sequence analysis of all 12 exons of the parkin gene revealed a novel G to A transition in exon 6 at position 736 (G736A) of the Parkin cDNA. This change results in a non-conservative cysteine for tyrosine substitution at residue 212 of Parkin. All affected individuals from two families were homozygous for this mutation, which was not detected in 100 normal controls from the same population. No mutation was identified in the family carrying the second haplotype, indicating that in this consanguineous pedigree the pathogenic change lies outside the coding region of parkin. This is the second founder effect that we demonstrate in this population. The concomitant genetic heterogeneity observed could result from recent immigration or might be related to the increased probability of survival of a young mutation in founder populations.

The evaluation of mutation analysis using DHPLC in CMT patients with MPZ, PMP22, GJB1, EGR2 mutations.

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Mutation detection occupies an important position in the clinical diagnosis of CMT patients. As disease-causing mutations have been identified in more and more genes, the labor and expense of screening for such mutations by nucleotide sequencing has increased substantially. Although techniques such as SSCP, DGGE are more economical, the sensitivity of these techniques is under 90% and a new method of screening for mutations is needed. Based on recent reports, Denaturing High-Performance Liquid Chromatography (DHPLC) appears to be an economical and highly sensitive method for mutation detection. We report optimal conditions for and the results of using DHPLC to identify mutations within some genes associated with CMT. We performed DHPLC on 50 patients with known mutations (8 with PMP22 mutations, 23 with MPZ mutations, 9 with GJB1 mutations, and 10 with EGR2 mutations) and controls using the Transgenomic Wave system. PCR reactions were performed using conventional hot start methods. Each coding exon was scanned (PMP22, 4 exons; MPZ, 6 exons; GJB1, 1 large exon split into 2 amplicons and EGR2, 2 exons split into 6 amplicons). The sequence alterations identified by DHPLC were confirmed by Dye Primer sequencing using an ABI Prism 377 sequencer. The PCR amplicons for DHPLC were prepared by slowly cooling from 95C to 25C over 45 min in order to form the heteroduplex. For males with GJB1 mutations, the heteroduplexes were formed as above after combining the PCR products from the patient with those from a normal control. We could identify the heterozygous mutations in each patient. The most important condition was the temperature of separation. Identification of mutations in patients with homozygous mutations is not possible and requires screening of the parents. In summary, DHPLC is an effective method for screening for heterozygous mutations in patients with CMT.

Autosomal dominant polycystic kidney disease (ADPKD): screening for mutations and polymorphisms in the duplicated region of the PKD1 gene. *A.E. Turco, S. Passigato, M. Fazon, F. Soli.* Department of Mother & Child, Section of Genetics, The University of Verona School Medicine, Verona - ITALY Italy.

ADPKD represents the most common potentially fatal inherited nephropathy (1/500 - 1/1000), and a leading cause of end stage renal failure in adults. ADPKD is genetically heterogeneous (at least three genes involved: PKD1, responsible for 80-90 percent of the cases, PKD2, and PKD3). The complex structure of PKD1 has thus far hindered mutation detection for most part of its span. However, methods have been developed to efficiently screen the duplicated region, both at the genomic or at the cDNA level. We used a long-range PCR approach to amplify a PKD1-specific genomic template spanning exons 23 through 34. Following an appropriate dilution step, this 10Kb fragment is then analyzed using exon-specific nested PCR reactions, and heteroduplex analysis (HA). Any putative mutation/polymorphism is then automatically sequenced (ABI Prism 377). The molecular confirmation and the segregation pattern of the mutation is carried out using restriction analysis. Currently, 155 ADPKD probands, in whom no mutations were identified in the 3' single copy region (exons 34-46), are being analyzed. To date the mutational screening has been almost completed in all the probands. Two single basepair deletions and several polymorphisms have been identified so far, indicating a low mutation detection rate. No changes were detected in exons 27, 32, and 33. The two frameshifting mutations were 9995/6 delT in exon 30, and 10089/90 delG in exon 31, both causing the same stop (M3395X) within the fourth transmembrane domain of polycystin 1. Both mutations abolish restriction sites, and thus allow easy testing in the probands' families. Other changes detected (pathogenic missense?) were: (GT)9195/9196(CC), exon 25, causing V3065V/F3066L, and G8440A, exon 23A, resulting in G2814R. Two frequent intronic polymorphisms were identified: A38944G, intron 24, detectable by RsaI, and G42447T, intron 28, detectable by StyI. These appear to be potentially useful tools for diagnostic linkage analysis. We suggest that a substantial number of disease mutations in these patients lie in the 5' region of the gene (exons 1-22).

C282Y and H63D hemochromatosis mutations prevalence in Brazilian HCV infected population. *C. Terra¹, L.C. Dolinsky², H.S.M. Coelho¹, J. Segadas¹, B.O.M. Vanderborght^{1,3}.* 1) Gastroenterologia, H.U.C.F.F., Univ Fed Rio de Janeiro, Rio de Janeiro, Brazil; 2) Dept Genetica, Inst Biologia, Univ Fed Rio de Janeiro, Rio de Janeiro, Brazil; 3) Innogenetics, Ghent, Belgium.

About 2% of the Brazilian population is infected with hepatitis C virus(HCV), which is the most common cause of hepatic transplantation in many countries. A lot of different factors can contribute to disease evolution, such as advanced age, iron overload, alcohol intake, etc. Iron overload can be due to hereditary hemochromatosis(HH), a disease caused by mutations in the HFE gene, that results on a higher iron absorption in colon cells followed by iron accumulation in different organs, such as heart, liver and pancreas. We studied in this work 150 patients infected with HCV. We did a search for two different hemochromatosis mutations: C282Y and H63D. To determine the patient genotype for each one of the mutations, we used PCR followed by restriction enzyme digestion. Our goal is to estimate the prevalence of the HH mutations in the Brazilian HCV infected population from Rio de Janeiro, a population composed of a mix from several ethnic groups. Data will be used in the near future to define the influence of the HH mutations on the clinical evolution of HCV infected patients. The frequency of the C282Y mutation found in this study was 4%. Homozygotes had a frequency of 0.7% and heterozygotes a frequency of 6.5%. H63D had a frequency about 3-times higher: the mutation frequency was 13%, with 2.2% of homozygotes and 22.30% of heterozygotes. We also found 2% of compound heterozygotes(C282Y/H63D). We can conclude, based on this data, that the frequencies of the hemochromatosis mutations found in the Brazilian population infected with hepatitis C are similar to that reported in literature. The ethnic mixture which is typical for the Brazilian population, did not influence the prevalence of both mutations under study.

A novel mutation in the C-propeptide coding region of *COL1A1* collagen gene results in osteogenesis imperfecta accompanied by osteopetrosis-like characteristics. *J.M. Pace¹, D. Chitayat², U. Schwarze¹, M. Atkinson¹, P.G.R. Seaward², P.H. Byers¹.* 1) University of Washington; 2) University of Toronto.

Osteogenesis imperfecta (OI) is characterized by brittle bones and is caused by mutations in the type I collagen genes, *COL1A1* and *COL1A2*. We identified a novel mutation in the C-terminal propeptide coding region of *COL1A1* that led to an unusual OI-like phenotype with features of osteopetrosis.

The newborn female had dysmorphic facial features, including loss of mandibular angle. Bilateral upper and lower limb contractures were present along with multiple fractures in the long bones and ribs. The ends of the long bones were radiographically dense. She died after a few hours. Autopsy revealed hepatosplenomegaly and extramedullary hematopoiesis in the liver. There was little lamellar bone formation and no osteoclastic activity. The shaft of the humerus and bony trabeculae were abnormally thick and marrow spaces were reduced, suggestive of osteopetrosis. Type I procollagen molecules synthesized by cultured dermal fibroblasts were overmodified along the full-length but had normal thermal stability. Sequence of cDNA identified heterozygosity for a 4321G→T transition in exon 52 that changed a highly conserved aspartic acid to a tyrosine (D1441Y). Pulse-chase studies showed that abnormal pro α 1(I) chains were slow to assemble into dimers and trimers and abnormal molecules were retained within the cell for an extended period.

Most mutations in the C-propeptide domains interfere with molecular assembly. In this instance assembly was delayed but triple helix structure appeared to be normal, given the normal stability. These findings suggest that the combined phenotype reflected both a diminished amount of secreted type I procollagen, and the presence of a population of stable and overmodified molecules that might support increased mineralization. Further, rare infants with osteopetrosis could have mutations in the C-terminal propeptide domain of type I procollagen that produce slow assembly and overmodified but stable molecules. (AR21557 and CA09437).

Novel mutations in the OPA1 gene and mutation detection in lymphocyte mRNA. *U.E.A. Pesch¹, C. Alexander², D.L. Thiselton², S. Mayer¹, B. Jurklies³, B. Leo-Kottler¹, U. Kellner⁴, M. Votruba², S.S. Bhattacharya², E. Zrenner¹, B. Wissinger¹.* 1) University Eye Hospital, Tuebingen, Germany; 2) Institute of Ophthalmology, University College London, UK; 3) University Eye Hospital, Essen, Germany; 4) University Eye Hospital Benjamin Franklin, Berlin-Steglitz, Germany.

Dominant optic atrophy of the Kjer type is the most frequent form of hereditary optic neuropathies with an estimated disease prevalence of 1:50000. Patients suffer from a progressive loss of visual acuity and color vision disturbances from early childhood onwards. Histological post-mortem examination of affected donor eyes suggested that the fundamental pathology results from a primary degeneration of the retinal ganglion cells followed by ascending atrophy of the optic nerve. Recently, we identified the gene responsible for autosomal dominant optic atrophy type Kjer (OPA1, OMIM 16500) on chromosome 3q28-q29. We found seven different mutations in families with dominant optic atrophy which segregate with the disease haplotype (Alexander et al. 2000, submitted). Here we describe the identification of additional new mutations in the OPA1 gene. This include two out of frame deletions of 4 bp and 22 bp, an complex deletion mutation and a putative splice site mutation. Most of the presently known OPA1 mutations result in a severely truncated polypeptide and support the hypothesis that dominant optic atrophy may result from haploinsufficiency. Due to the complex structure of the OPA1 gene, mutation analysis based on the analysis of single exons amplified from genomic DNA is cumbersome and time consuming, and did not allow to detect larger deletions. We therefore isolated total RNA from white blood cells of OPA1 patients and amplified four overlapping OPA1 cDNA fragments by means of RT-PCR. PCR products were purified and sequenced with the PCR primers. Preliminary analysis indicate that at least some mutant alleles are expressed - albeit at reduced level - in lymphocytes, bearing the potential for rapid and effective mutation screening of the OPA1 gene.

Characterization of two retinoschisis gene mutations: a noncontiguous two-part deletion and a deletion covering exons of three adjacent genes RS1, PPEF1, and STK9. *H. Tyynismaa*^{1,2}, *L. Huopaniemi*^{1,2}, *J. Tommiska*^{1,2}, *A.*

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X-linked juvenile retinoschisis (RS) is a recessively inherited disorder causing progressive vitreoretinal degeneration with variable degrees of severity. The gene defective in retinoschisis, RS1, has recently been identified and more than 100 mutations have been published. Single base substitutions clearly dominate the mutation spectrum. Only a few single exon deletions have been described. We have now studied in detail two RS1 gene mutations, originally described as exon 1 deletions, segregating in Danish families. The smaller of the deletions covers 4.4kb and extends from the 5' flanking region of the retinoschisis gene to RS intron 1. The mutation is a noncontiguous two-part deletion composed of deleted 1.5kb and 2.9kb segments, separated by an intact 1.2kb segment. STS mapping and Southern blotting revealed that the larger of the two deletions covers a total of 136kb, spanning from the 5' region of the RS1 gene to intron 3. Deletion breakpoints are located in the middle of Alu repeats. Because the RS1 gene is partly overlapping with STK9 (serine/threonine kinase) gene, in tail to tail position, the 136kb deletion also abolishes the last exon of STK9. In addition, the PPEF1 (protein phosphatase) gene, which is located 5' to RS1, is affected by this mutation. It deletes the putative promoter region and exons 1-9 of PPEF1. Since the direction of transcription in STK9 and PPEF1 is the same and the deletion localizes the 3' end of STK9 exon 19 to only 2.8kb distal to the exon 10 of PPEF1, it is possible that this rearrangement leads to a fusion protein. The N-terminus of the new protein would represent STK9 and the C-terminal part PPEF1. Despite of the unique deletions, all the RS patients have a typical retinoschisis phenotype.

Maternal transmission of 3 bp deletion in exon 7 of the STS gene in X-linked ichthyosis. *M. Valdes*¹, *A. Jimenez*², *S. Kofman*², *S. Cuevas*². 1) Genetica, Instituto Nacional de Ortopedia; 2) Genetica, Hospital General de Mexico, Fac. Medicina, UNAM.

Steroid sulfatase (STS) deficiency is an inherited error of metabolism causing X-linked ichthyosis (XLI). Onset is at birth or early after birth and it is clinically characterized by dark, regular, and adherent scales of skin. STS is a microsomal enzyme with ubiquitous tissue distribution that hydrolyzes 3-beta-steroid sulfates. Approximately 85-90% of XLI patients have large deletions of the STS gene and flanking sequences. Only few point mutations have been observed. Seven patients have been reported with partial deletions of the STS gene, these include: a deletion within intron 7 extending over exons 8-10; a partial deletion that include exon 10; an intragenic deletion involving exons 2-5; a deletion of exons 2-10 spanning within intron 1 and between flanking sequences DXS1131 and DXS1133. It has also been also reported two unrelated cases with partial deletions of the STS gene and its centromeric region and one patient in whom the telomeric end of the extragenic segment was partially missing. In this study, we describe a different partial deletion of the STS gene in an XLI patient that was inherited from his mother. The subject and his mother were classified through STS assay in leukocytes using 7-[3H]-dehydroepiandrosterone sulfate as a substrate. Exons 1, 2, 5, 7-10 of the STS gene were analyzed through PCR and DNA sequencing. STS activity was undetectable in the XLI patient (0.00 pmol/mg protein/h) and very low in his mother (0.32 pmol/mg protein/h vs 0.84 pmol/mg protein/h of normal control). DNA analysis showed a 3 bp deletion (1252AAG) within exon 7 of the STS gene in the XLI patient and heterozygote state for this deletion in his mother. This finding corresponds to the first 3 bp intragenic deletion of the STS gene causing XLI. This data shows the diversity of molecular defects of the STS gene observed in XLI.

Two mutations in a patient with dopa-responsive dystonia. *M.K Paik¹, Y.S. Kim², K.H. Cho², K.M. Hong¹.* 1) Biochemistry, Wonkwang University College of Medicine, Iksan, Korea (ROK); 2) Neurology, Wonkwang University College of Medicine, Iksan, Korea (ROK).

Dopa-responsive dystonia (DRD) is an autosomal dominant, childhood onset postural dystonia characterized by diurnal fluctuation and response to L-dopa. This disease is caused by mutations in the gene encoding GTP cyclohydrolase I (GCH1) that is the first enzyme in the pathway converting GTP to tetrahydrobiopterin. The GCH1 gene mutations were also found in recessively inherited atypical hyperphenylalaninemia which has different clinical symptoms and disease course from DRD. A 10 year-old male patient who has been walking problem since 8 year old showed diurnal fluctuation of the dystonia and responded to L-dopa, which are typical DRD symptoms but different from those of atypical hyperphenylalaninemia. His plasma levels of phenylalanine and tyrosine were within normal range when he was admitted. He has no family history of similar dystonia. Analysis of sequences from PCR amplification of the GCH1 exons including exon/intron junctions revealed two novel mutations, R59G and R198W. His mother had R59G and his father had R198W mutation. These data suggest that dopa-responsive dystonia might also be inherited in an autosomal recessive way.

Gene dosage using CALD analysis reveals complex rearrangements in two DMD families. *L. Steele¹, C.A. Ryan¹, E. Lemire³, P.N. Ray^{1,2}*. 1) Dept Pediatrics Lab Med, The Hospital for Sick Children, Toronto, ON, Canada; 2) Genetics & Genomic Biology, The Hospital for Sick Children, Toronto, ON, Canada; 3) Div Medical Genetics, Royal University Hospital, Saskatoon, SK, Canada.

Duchenne Muscular Dystrophy (DMD) is an X-linked condition with an incidence of 1 in 3,300 male births. Approximately 60% of the DMD mutations are due to a deletion within the gene while it is estimated that an additional ~10% are due to a segmental duplication. The mechanism by which DMD deletions and duplications arise is believed to be distinct. Computer-Assisted Laser Densitometry (CALD) is used to examine the gene dosage of 18 DMD exons and the brain promoter and has previously been shown to have a significant impact on assigning a more definitive carrier status in women at risk for DMD. Here we report two DMD families that illustrate the importance of performing gene dosage using CALD analysis. The first case is of a manifesting carrier of DMD with findings of muscle weakness, abnormal dystrophin staining on muscle biopsy and an elevated CK. CALD analysis of two independent samples from the proband revealed a duplication of exons 45 & 48 with normal dosage for exon 47 of the DMD gene. Subsequent analysis of her mother's DNA revealed a deletion of exon 47 in the DMD gene. These results suggest that the original DMD mutation was in fact a deletion of exon 47, which was converted to a complex duplication allele during meiosis through misalignment between homologous chromosomes followed by unequal crossing over distal to exon 45 of the normal DMD gene. A similar mechanism is postulated for the second DMD family of a mother and three daughters with an extensive family history of DMD. The mother and one of her daughters, who were reported to have elevated CK, were found by CALD analysis to have a complex duplication involving exons 45-50 and 60, while they had normal dosage for exons 51-52. The two daughters with normal CKs were found to have normal dosage for all DMD exons. Results of linkage studies in both cases will also be presented. These studies suggest that there is a potential of finding different mutations within a family due to the high rate of recombination observed in DMD families.

Role of complex alleles and gender in susceptibility to Familial Mediterranean Fever in the Armenian

population. *Y. Torosyan*¹, *I. Aksentijevich*¹, *T. Sarkisian*², *H. Ajrapetyan*², *G. Amaryan*³, *V. Astvatsatryan*³, *D.L. Kastner*¹. 1) NIAMS/NIH, Bethesda, MD; 2) Center of Medical Genetics, Yerevan, Armenia; 3) Yerevan State Medical University, Armenia.

Familial Mediterranean Fever (FMF) is an autoinflammatory disorder characterized by self-limited episodes of fever and localized inflammation, caused by recessively inherited mutations in the MEFV gene. Here we report the results of an ongoing survey of MEFV mutations in Armenian FMF patients and controls. Among 288 independent chromosomes from healthy Armenian controls, the total carrier frequency was 0.37, whereas the carrier frequency for exon 10 mutations was 0.25. We found MEFV mutations in 93% of 278 independent carrier chromosomes from 214 Armenian FMF patients, including a novel mutation (E230K) in exon 2. Of the patients' chromosomes on which mutations could be found, only 9% had non-exon 10 mutations. The discrepancy between the frequency of non-exon 10 mutations among patients and in the general population suggests that penetrance is reduced for mutations outside of exon 10. Moreover, we identified 43 individuals without symptoms of FMF who had mutations on both chromosomes, and almost half of the 86 alleles bore either E148Q (exon 2) or P369S (exon 3). Among 10 genotypes with P369S complex alleles, 7 were not associated with clinical FMF, and the other 3 manifested only mild disease, suggesting that P369S might actually ameliorate the phenotypic effects of exon 10 mutations in cis. In addition, gender appeared to affect the penetrance of genotypes bearing the exon 10 mutations M680I (39M/23F, $P < 0.001$) or V726A (37M/22F, $P < 0.01$) on either chromosome, whereas genotypes bearing M694V (which is also in exon 10) were distributed equally (52M/45F, NS). Taken together, these data indicate a complex interaction of genetic determinants within and distinct from MEFV in determining phenotypic severity in FMF. *MEFV cis*.

Mutational analysis of *GJB2* in an unselected deaf population. *E.M. Rohlf*¹, *M. Zariwala*², *J.K. Booker*¹, *M.I. Roche*³, *C.M. Powell*³, *L.M. Silverman*¹, *C.G. Shores*⁴. 1) Dept Pathology & Laboratory Med; 2) Cystic Fibrosis Center; 3) Dept Pediatrics; 4) Division ENT Surgery, Univ North Carolina, Chapel Hill, NC.

Approximately 50% of recessive nonsyndromic hearing loss is due to mutations in the *GJB2* gene (connexin 26) and a single base pair deletion at nucleotide 35 accounts for between 70% and 85% of the disease-causing mutations. We sequenced the coding region of the *GJB2* gene in 66 deaf individuals (63 families) who presented to the ENT clinic for evaluation of hearing loss or evaluation for a cochlear implant. 57 of the individuals were Caucasian, 4 African-American, 2 Hispanic, 2 Asian/Pacific Islander and 1 Native-American. 7 (12%) Caucasians were homozygous for a disease causing mutation (6, 35delG homozygous; 1, W24X homozygous). Four (7%) Caucasians were heterozygous for 35delG but an additional mutation was not detected despite full sequencing. This carrier frequency is 3.5 times higher ($p=0.045$) than the carrier frequency in the North Carolina Caucasian population (2%) and suggests the possibility of other mutational mechanisms in *GJB2* (i.e. methylation, promoter mutation, gene rearrangement) or the interaction of the connexin 26 protein with other related proteins that may carry a mutation (i.e. other connexins). The sequence variant/mutation M34T, was detected in 2 deaf individuals, one of whom was Native American. Neither individual reported a family history of deafness, ruling out a completely penetrant autosomal dominant form of inheritance. Two novel sequence variants, his73arg and arg184gln, were detected in Caucasian individuals. The mutation of arginine 184 to both proline and tryptophan are known to be disease causing, however the functional consequence of the substitution with a glutamine is not known. All individuals were also tested for the 12S rRNA mitochondrial gene mutation A1555G, and were negative. Screening for mutations in *GJB2* in an unselected deaf population can provide a genetic diagnosis for a significant proportion of the individuals. However, the interpretation of heterozygous genotypes remains problematic. In addition, the etiology of deafness may become an important predictor of clinical outcome of cochlear implants.

KvLQT1 Mutations Associated with Cardiac Arrhythmia and Sudden Death Triggered by Sports. *Q. Wang¹, F. Zimmerman², P. Szafranski³, R.M. Bryant⁴, C. Oberti⁵, M. Flippin², D. Marangi⁴, R. Sterba¹, A. Strauss², P. Tchou¹, M. Chung¹.* 1) The Cleveland Clinic Foundation, Cleveland, OH 44195; 2) Washington University School of Medicine, St. Louis, MO; 3) Baylor College of Medicine, Houston, TX; 4) University of Florida Health Science Center, Jacksonville, FL; 5) School of Medicine, Montevideo, Uruguay.

Long QT syndrome (LQT) is a cardiac rhythm disorder that is characterized by prolongation of QT interval on electrocardiograms. When stimulated by triggering factors, some LQT patients will develop symptoms such as syncope, seizures, a specific polymorphic ventricular arrhythmia known as torsade de pointes, and sudden death. However, the molecular nature of the triggering factors is largely unknown. Five LQT genes have been identified: the cardiac sodium channel gene SCN5A (LQT3) on chromosome 3p21, and cardiac potassium channel genes KvLQT1 (LQT1) on chromosome 11p15.5, HERG (LQT2) on chromosome 7q35-36, KCNE1 (LQT5) and KCNE2 (LQT6) on chromosome 21q22. We have characterized a large family in which LQT-associated arrhythmia and sudden cardiac death were stimulated by swimming. Linkage analysis with markers covering the known LQT genetic loci suggested that the LQT-causing mutation in the family is located within KvLQT1. SSCP and DNA sequence analyses revealed a G174S mutation in transmembrane domain S5 of KVLQT1 in all affected individuals, but not in unaffected individuals. In one small family in which LQT-associated symptoms were stimulated by basketball-playing, the affected individuals carry a Y220C mutation in the pore domain of KVLQT1. Based on these data, we conclude that KvLQT1 mutations cause cardiac arrhythmia and sudden death associated with sports. (This study was supported by the Cleveland Clinic Foundation Seed Grant and the American Heart Association).

Linkage studies and mutation analysis of SPINK5 in an international cohort of 24 families with Netherton

syndrome. *G. Richard*¹, *E. Sprecher*¹, *S. Chavanas*², *C. Miller*¹, *S.J. Bale*³, *J.J. DiGiovanna*^{3,4}, *A. Hovnanian*², *J. Uitto*¹. 1) Dermatology & Cutaneous Biol, Thomas Jefferson Univ, Philadelphia, PA; 2) Wellcome Trust Centre for Human Genetics, Oxford UK; 3) NIAMS, NIH Bethesda, MD; 4) Brown University/Rhode Island Hospital Providence, RI.

Netherton Syndrome (NTS) is a rare, autosomal recessive disorder characterized by congenital ichthyosis, hair shaft abnormalities and immune deficiency associated with recurrent infections and highly elevated IgE levels. To delineate the spectrum of clinical features and elucidate the genetic basis of NTS, we ascertained 24 unrelated families of various ethnic background. Using multipoint linkage studies in 12 families with multiple siblings, we excluded 3 candidate gene loci and confirmed linkage of NTS to 5q32, where the NTS locus recently has been mapped (Chavanas et al., *Am J Hum Genet*, 66:914-921, 2000). Analysis of 4 microsatellite markers spanning 12 cM revealed complete co-segregation between the disease and 2 marker loci with a maximum LOD score of 5.1 at D5S434. These data provide strong evidence for genetic homogeneity in NTS across families of different origin. The NTS critical region harbors SPINK5, a gene encoding the multidomain serine proteinase inhibitor LEKTI, which is predominantly expressed in epithelial and lymphoid tissues. Recently, Chavanas et al. (*Nature Genet* 25:141-142, 2000) reported mutations in SPINK5 as cause of NTS. Screening of our cohort using CSGE followed by direct automated sequencing thus far revealed 15 distinct mutations, all of which are novel and predicted to lead to premature termination of protein translation. The majority of mutations (n=9) were small deletions/insertions resulting in frameshift, 3 mutations altered a splice site, and 3 mutations introduced a premature stop codon. Four different mutations clustered in exon 5, while the remaining were scattered across exons 3 through 27. In addition, we identified 7 silent polymorphisms of the coding sequence of SPINK5. Our results implicate LEKTI in epidermal differentiation, barrier function, and immune response, and provide the basis for defining potential phenotype-genotype correlations in NTS.

ALELLIC AND LOCUS HETEROGENEITY IN AUTOSOMAL RECESSIVE GELATINOUS DROP-LIKE CORNEAL DYSTROPHY. Z. Ren¹, P-Y. Lin¹, G.K. Klintworth², F.L. Munier³, D.F. Shorderet³, L. El Matri⁴, M.I. Kaiser-Kupfer¹, J.F. Hejtmancik¹. 1) National Eye Inst, NIH, Bethesda, MD; 2) Departments of Pathology and Ophthalmology, Duke University Durham, NC USA; 3) Oculo-Genetic Unit, Lausanne University, Lausanne, Switzerland; 4) Institut Hedi Rais d'Ophtalmologie de Tunis, Tunisia.

Autosomal recessive gelatinous drop-like corneal dystrophy (GDLD, also named familial subepithelial corneal amyloidosis) is a rare disease characterized by the deposition of amyloid in the cornea and by the severely impaired visual acuity leading to blindness. Tsujikawa et al. (1998, 1999) previously have mapped gelatinous corneal dystrophy to chromosome 1p and shown that it is associated with mutations in the M1S1 gene. A total of 11 affected families were collected for linkage analysis. In these families the disease locus was mapped between markers D1S519 and D1S2835 to a 16 cM interval on the short arm of chromosome 1 including M1S1 gene. To investigate the molecular genetic basis of this disease, a 1.2 kb fragment containing entire coding region of M1S1 gene was amplified by PCR from genomic DNA and subsequently sequenced. Eight different mutations among 10 families were identified. These mutations include an 8-base insertion at nt 782, single base deletions at nt 867delC and nt 1117delA, single base substitutions at nt T308G, T661A, C658G, C658T and T887A. These mutations cause reading frame shift, a premature stop codon and substitution of amino acids. No abnormal sequence was detected in a single family, which was also excluded from this locus by linkage analysis. Our findings suggest that heterogeneous mutations of M1S1 cause GDLD in these families and multiple loci may be involved in GDLD.

Exclusion of the Elastin gene in the pathogenesis of Costello syndrome. *C. Tandoi¹, A. Botta¹, G. Fini¹, F. Sangiuolo¹, R. Ricci², G. Zampino², C. Anichini³, A. Giannotti⁴, B. Dallapiccola⁵.* 1) Dept. Biopathology, "Tor Vergata" University, Rome, Italy; 2) Catholic University, Rome, Italy; 3) University of Siena, Siena, Italy; 4) Dept. Medical Genetics, "Bambino Gesù"; Hospital, Rome, Italy; 5) "La Sapienza" University of Rome and CSS-Mendel Institute, Rome, Italy.

Costello syndrome is a rare congenital disorder characterised by post natal growth and mental deficiency, coarse facies, loose skin of the neck, hands and feet, cardiomyopathy and nasal papillomata. Major histological changes include fine, disrupted elastic fibers in the skin, tongue, pharynx, larynx, and arteries, suggesting an involvement of elastin (ELN) protein in the pathogenesis of the disorder. In order to verify this hypothesis, we searched for mutations within the entire coding sequence and exon boundaries regions of the ELN gene in four patients with Costello syndrome. PCR products containing each exon of the ELN gene were amplified by genomic DNA of our patients using intronic primers and examined by combined single strand conformation polymorphism (SSCP) and direct sequencing of aberrant conformers. No pathogenic mutations were detected. We did however found a novel G -> C polymorphism localized to nucleotide 1828, resulting in an amino acid change of Gly Arg (G/R610) in codon 610 of the ELN gene, which predicted the substitution of Gly (GGG) for Arg (CGG) at this position. This is the first mutation screening of the ELN gene in patients with Costello syndrome. Our results exclude the direct involvement of this gene in the pathogenesis of the disorder. Work supported by Ministero della Sanità.

Missense mutations: expansion of the phenotype of laminin a2 deficiency. Z. Tezak¹, J. Devaney², M. Marino², E. Pegoraro³, R. Finkel⁴, E.P. Hoffman¹. 1) CRI III, CNMC, Washington, DC; 2) Transgenomic, Inc., Gaithersburg, MD; 3) University of Padova, Clinica Neurologica, Padova, Italy; 4) Division of Neurology, The Children's Hospital of Philadelphia, Philadelphia, PA.

Complete laminin a2 deficiency causes approximately half of congenital muscular dystrophy (CMD) cases. Many loss-of-function mutations have been reported in these severe, neonatal onset patients, but only single missense mutation in milder CMD has been published so far. Here, we studied 18 patients diagnosed with CMD who displayed partial deficiency of laminin a2 on immunofluorescence of muscle biopsy. We screened the entire 9.5kb laminin a2 mRNA from patient muscle biopsy by either direct capillary automated sequencing, or denaturing high performance liquid chromatography (DHPLC) of overlapping RT-PCR products followed by direct sequencing of heteroduplexes. We identified causative mutations in 9 of 18 patients, suggesting primary laminin a2 gene defect in about half of partial laminin a2 deficiency patients. Each of the gene changes identified was a novel mutation, including 4 missense mutations: Cys862Arg, Asn1099Ser, Pro2925Leu, and Thr2636Ala, all evolutionary conserved residues. Cys862 and Asn1099 are located in rigid rod-like domain domain IIIb. Pro2925 and Thr2636 are located in G-domain, predicted to participate in the binding of the laminin a2 to the membrane receptors. In one patient, we found a G to A transition at position +5 of the conserved donor splicing consensus sequence of intron 37, which led to a deletion of a complete exon involved in triple coiled-coil structure with laminin b1 and g1. We also found 6 out-of-frame heterozygous deletions introducing premature stop signals. Clinical phenotype of CMD patients with partial laminin a2 deficiency is usually milder, with some reported cases of epilepsy, rare noted in completely laminin a2 deficient patients. One of the patients in whom we identified disease causing missense mutation had unique cystic lesions within white matter, suffered from epilepsy and seizures, and showed progressive dementia. This report expands the phenotypes of laminin a2 deficiency.

Mutation Analysis in Dominant Drusen: Direct Sequencing of EFEMP1. *S. Sheikhavandi, N. Udar, P. Vyas, A. Shirvanian, S. Yelchits, H. Nugyen, R. Garcia, K. Small.* Department of Ophthalmology, UCLA School of Medicine, Los Angeles, CA.

Dominant Drusen is an autosomal dominant disorder characterized by yellow-white deposits known as drusen that accumulate beneath the retinal pigment epithelium. The Dominant Drusen locus was mapped to chromosome 2p16-21. EFEMP1 was recently identified as a candidate gene causing Dominant Drusen. Different mutations of EFEMP1 have thus far been identified with a single non-conservative mutation of Arg345Trp accounting for 90% of all the disease causing mutations. In this study, we have evaluated the EFEMP1 gene in 10 unrelated families with Dominant Drusen. A mutation screening of the coding region of the EFEMP1 gene was carried out by direct sequencing of PCR products. We examined 12 exons and flanking intron/exon boundaries in patients with Dominant Drusen phenotype along with normal controls. Several polymorphisms were detected. 2 families showed an A>G change in exon 5, 2 families and the control showed a one nucleotide deletion in exon 12, 2 families showed an A>G change in exon 6 and 3 families showed an intronic change of C>T in intron 10. However, none of our families had any coding sequence or splice site mutations and none showed the Arg345Trp mutation previously observed in the EFEMP1 gene.

Mutation Analysis of IMPG1 gene in MCDR1 families. *S. Yelchits¹, N. Udar¹, B. Puech², S. Sheikhavandi¹, L. Morales¹, H. Nugyen¹, R. Garcia¹, K. Small¹.* 1) Department of Ophthalmology, Jules Stein Eye Inst, University of California, Los Angeles, Ca; 2) Clinique Ophthalmologique, Centre Hospitalier Regional Universitaire De Lille, France.

Interphotoreceptor matrix proteoglycan-1 (IMPG1), selectively expressed in retinal tissue, is thought to play a critical role in the function of photoreceptor cells. Localization of IMPG1 at chromosomal region 6q13-q15 made this gene an attractive candidate for 6q-linked retinal dystrophies, including North Carolina Macular Dystrophy (MCDR1). MCDR1 is an autosomal dominant macular dystrophy causing impaired central vision at an early age. Mutation analysis was carried out on eleven MCDR1 families with different descent and haplotypes. To increase sensitivity of mutation detection all samples were checked using both Single Stranded Conformation Polymorphism (SSCP) and dHPLC. We found base changes in exons 2, 13, 14, 15 & 16; but these did not segregate with the disease. We detected one base pair variation in intron 8 of all the affected members in four generation french family, which segregate with the disease. Further studies are necessary to characterise this base change.

A PCR-sequencing based approach for clinical molecular diagnosis in the Doublecortin gene. *B.L. Wu¹, V. Lip¹, J. Gleeson², C.A. Walsh³, B.R. Korf⁴*. 1) Dept of Laboratory Medicine and Pathology, Children's Hospital and Harvard Medical School, Boston, MA; 2) Dept of Neuroscience, Univ of California San Diego, La Jolla, CA; 3) Dept of Neurology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; 4) Paterners Center for Human Genetics and Dept of Neurology, Harvard Medical School, Boston, MA.

X-linked double cortex and lissencephaly syndrome (OMIM 300067) is an uncommon disorder with a dosage-related phenotype. Affected males have intractable seizures, severe mental retardation, growth failure, lissencephaly (smooth brain), and die during infancy. Hemizygous females show a milder phenotype, known as subcortical laminar heterotopia or double cortex, which is characterized by milder mental retardation, milder epilepsy, and survival into reproductive life. Diagnosis is usually inferred by clinical presentation and confirmed by autopsy before the Doublecortin gene (DCX) was cloned (des Portes V and Chelly J et al. *Cell*, 92:51-61, 1998; Gleeson JG and Walsh CA et al. *Cell*, 92:63-72, 1998). A PCR-sequencing based approach for the detection of double cortex mutations in the DCX gene has been established, which is useful to confirm the clinical diagnosis of double cortex. Each of the six exons of the gene is amplified by PCR and sequenced using an automated fluorescence DNA sequencer. A proficiency test was first done using a set of previously characterized mutations from ten unrelated patients, including deletions, missense and nonsense mutations. All the previously identified mutations were found. Total of 16 patients including one prenatal samples have been studied using this approach. Five different mutations were detected.

GJB2 GENE A NOVEL MUTATION FOUND IN PORTUGUESE DEAF FAMILIES. *H. Vieira¹, M. Vitorino¹, J. Camara¹, A. Mena¹, H. Caria¹, M. Simao², I. Galhardo², T. Netta², O. Dias², M. Andrea², C. Correia¹, G. Fialho¹.* 1) Centre of Genetics and Molecular Biology, University of Lisbon, Portugal; 2) Centre of ORL, University of Lisbon, Portugal.

Mutations in GJB2 gene, encoding connexin 26, have been shown as a major contributor to prelingual, sensorineural, nonsyndromic recessive deafness (NSRD) in Caucasian populations. One specific mutation, 35delG, is the most prevalent mutation in this gene, and one of the most frequent disease mutations identified so far. In the present study we report a screening for GJB2 mutations performed in 55 individuals from 10 NSRD families, using PSDM, SSCP and direct sequencing approaches. As we have reported previously, half of the families were positive for 35delG, being 40% homozygous and 60% heterozygous. In the heterozygous and negative cases, analysis of the complete coding region as well as of the splice junctions of the GJB2 gene, revealed the presence of an additional known mutation, M34T, and a novel splicing mutation, IVS123G-T. This novel mutation is just the second splicing mutation described for the GJB2 gene, and is predicted to lead the formation of an abnormal mRNA. The M34T mutation was found in compound heterozygosity with the IVS123G-T mutation, supporting the hypothesis that M34T by itself cannot cause deafness but together with another mutation can alter the hearing phenotype. Molecular detection of Cx26 mutations in portuguese affected families will provide early diagnosis and prognostic thus representing a considerable aid in genetic counselling.

Diminished levels of EXT1 and EXT2 tumor-suppressor proteins in exostosis and in chondrosarcoma cell lines.

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The *EXT* genes belong to a family of putative tumor suppressor genes that affect endochondral bone growth. Mutations in *EXT1* and *EXT2* genes cause the autosomal dominant disorder Hereditary Multiple Exostoses (HME), and loss of heterozygosity (LOH) of these genes plays a role in the development of exostoses and chondrosarcomas. The *EXT* genes are expressed ubiquitously, yet abnormal bone growth is the only apparent consequence of mutations in *EXT1* and *EXT2* genes.

In this study, we characterized *EXT* genes in 11 exostosis chondrocyte lines using LOH and mutational analyses. In addition, we raised antibodies against unique epitopes of the EXT1 and EXT2 proteins and characterized these EXT proteins in 5 control costochondral chondrocyte, 1 fibroblast, 32 exostosis chondrocyte and 7 chondrosarcoma cell lines by immunoperoxidase staining. Both EXT proteins have peri-nuclear localization in control chondrocytes, fibroblasts, exostosis chondrocytes and chondrosarcomas. EXT1 and EXT2 protein levels are decreased in 21 (66%) and 15 (47%) of 32 exostosis chondrocyte lines, respectively. Levels of EXT1 and EXT2 proteins were diminished in 71% and 57% of 7 chondrosarcoma lines, respectively. Although we found LOH and mutations in exostosis chondrocytes, mutational analysis alone did not predict all the observed decreases in *EXT* gene products in exostosis chondrocytes, suggesting additional genetic mutations.

Other studies have shown that EXT1, EXT2 and the Drosophila homolog EXT-D/Ttv are glycosyltransferases responsible for heparan sulfate biosynthesis of HS proteoglycans and that EXT-D/Ttv promotes inter-cellular diffusion of the growth factor Hedgehog. Taken together, these results suggest that exostoses and chondrosarcomas are abnormal in EXT1 and/or EXT2 proteins levels, which is expected to lead to defective cell signaling, perhaps in the Hedgehog pathway.

Pathological mutations of tuberin affect interaction with hamartin. *A.K Hodges¹, J. Maynard¹, L. Parry¹, J.E DeClue², J. Cheadle¹, J. Sampson¹.* 1) Institute of Medical Genetics, UWCM, Cardiff, United Kingdom; 2) Laboratory of Cellular Oncology, NIH, Bethesda, USA.

The functions of hamartin and tuberin in the cell are likely to be closely linked: the tuberous sclerosis phenotypes associated with TSC1 and TSC2 mutations are very similar, the proteins appear to co-localise at the cellular level, and biochemical evidence suggests that hamartin and tuberin are able to interact directly with each other. However, the regions of hamartin and tuberin that interact are not well defined, and the relationship between their interaction and the tuberous sclerosis phenotype has not been explored. In order to define the boundaries of hamartin and tuberin interaction, a series of hamartin and tuberin constructs in activating and binding domain vectors were constructed to assay for interaction using the yeast two-hybrid assay. Tuberin (aa 1-418) and hamartin (aa 302-430) interacted strongly with each other. In contrast to previous reports, the large coiled-coil region of hamartin (aa 719-998), whilst capable of oligomerization, was not found to be important for tuberin interaction. A putative coiled-coil region of tuberin (aa 346-371) was necessary but not sufficient to mediate interaction with hamartin. Yeast two-hybrid constructs of this minimal binding region of tuberin that incorporated tuberous sclerosis associated missense (294G>E) and in-frame deletion (365delI) mutations were produced by site-directed mutagenesis and by RT-PCR from patient-derived RNA. While the wild-type tuberin construct interacted strongly with hamartin, the mutant proteins failed to interact. Furthermore, four non-pathogenic missense polymorphisms (R261W; M286V; R367Q; P378L) flanking the disease associated mutations did not abolish the interaction. These results show for the first time a link between the pathogenesis of tuberous sclerosis and the coupling of hamartin and tuberin. The area in hamartin that interacts with tuberin overlaps with a putative Rho activating domain. Exploitation of TSC associated natural mutants will help to establish the relationship between hamartin and tuberin binding, Rho activation, and the pathogenesis of tuberous sclerosis.

A missense mutation at the g42 secretase site in APP points to an essential role for N- truncated Ab42 in Alzheimer's disease. *C. Van Broeckhoven¹, S. Kumar-Singh¹, C. De Jonghe¹, M. Cruts¹, R. Kleinert², R. Wang³, M. Mercken⁴, H. Vanderstichele⁵, A. Löfgren¹, E. Vanmechelen⁵, P.M. Kroisel².* 1) Laboratory of Molecular Genetics, Flanders Interuniversity Institute for Biotechnology (VIB), Born-Bunge Foundation (BBS), University of Antwerp (UIA), Department of Biochemistry, Antwerpen, Belgium; 2) University of Graz, Graz, Austria; 3) The Rockefeller University, New York, USA; 4) Janssen Research Foundation, Beerse, Belgium; 5) Innogenetics Inc., Zwijnaarde, Belgium.

Amyloidogenic processing of the amyloid precursor protein (APP) with deposition in brain of the 42 amino acids long amyloid b-peptide (Ab) is considered central to Alzheimer's disease (AD). Maturation by Ab40 of nonfibrillar Ab42 into fibrillar amyloid plaques causes neurodegeneration. Amyloidogenic processing of APP involves cleavage by b-secretase followed by g40- (Ab40) or g42-secretase (Ab42) cleavage, generating full-length Ab. In contrast constitutive processing of APP involves cleavage within Ab by a-secretase producing N- truncated Ab40 or Ab42 (p3). We identified a novel missense mutation (Thr714Ile) directly involving g42-secretase cleavage of APP in an Austrian AD family with extreme early onset age (mean onset age 35 years) and rapid progression of disease. Modeling the mutation in transiently transfected HEK293 cells and analyzing the conditioned medium by ELISA and MALDI-TOF mass spectrometry, indicated that Thr714Ile has the most drastic effect on Ab secretion with a 10-fold increased Ab42/Ab40 ratio, simultaneously increasing Ab42 and decreasing Ab40. A similar effect was observed in patient's plasma. In brain this coincided with deposition of abundant and predominant nonfibrillar pre-amyloid plaques composed primarily of N-truncated Ab42 in the complete absence of Ab40. This indicates that N-truncated Ab42 as diffuse nonfibrillar plaques has an essential role in AD pathology. Consequently, one should keep in mind that inhibiting secretion of full-length Ab42 by therapeutic targeting of b- and/or g-secretases, might switch to increased utilization of the a- secretase pathway with the production of N-truncated Ab42 which is potentially as toxic as full-length Ab42.

Tissue mosaicism in two mildly affected PBD patients with identical PEX1 mutations. *J. Gartner¹, S. Weller¹, N. Preuss¹, U. Brosius¹, W. Schmitz², E. Conzelmann².* 1) Pediatrics, Heinrich Heine University, Dusseldorf, Germany; 2) Biological Chemistry, Julius Maximilian University, Wurzburg, Germany.

The peroxisome biogenesis disorders (PBD) are a group of lethal autosomal recessive diseases with overlapping clinical phenotypes including Zellweger syndrome, neonatal adrenoleukodystrophy and infantile Refsum disease. Their defects are caused by mutations in different PEX genes. The cellular hallmark are the failure to assemble normal peroxisomes and the concomitant loss of multiple peroxisomal enzyme activities. These defects are assumed to be expressed in all cells, and the diagnostic analyses as well as research work are based on this assumption. We describe two male patients (10 and 23 years of age) with the characteristic clinical, biochemical and molecular features of PBD. However, the peroxisome defect of these patients is not expressed in all cells. Immunofluorescence and electron microscopy studies in fibroblasts and liver revealed peroxisome deficient cells adjacent to normal cells indicating mosaicism. Both patients are within complementation group 1 and have two compound heterozygous mutations in the PEX1 gene: G843D and c.1960-1961insCAGTGTGGA. Mutations in the PEX1 gene are responsible for more than 50% of all PBD cases. In patients homozygous for the G843D allele a mild clinical course can be expected. In contrast, insertions in the PEX1 gene are known to cause severe forms of disease. The origin of the mild clinical course in these two mosaic PBD patients might be residual activity of the mutant PEX1 alleles, complementation of the different mutant alleles or the peroxisomal mosaicism. The latter is most likely and the mosaicism might be due to reversion of the PEX1 mutation in one allele or somatic loss of heterozygosity in fetal development. Molecular analyses of peroxisome deficient and normal cells are in progress.

Construction and analysis of a cosmid and BAC clone contig covering the Duane critical region on chromosome

8q13. *G. Calabrese*¹, *A. Pizzuti*², *L. Telvi*³, *E. Morizio*¹, *M. Bozzali*², *A. Ratti*², *D. Fantasia*¹, *F. Capodiferro*¹, *V. Gatta*¹, *A. Ion*³, *L. Stuppia*¹, *G. Palka*¹, *B. Dallapiccola*⁴. 1) Sez. Genetica, Dip. Scienze Biomediche Univ DG Annunzio, Chieti, Italy; 2) Cl. Neurologica, Policlinico, Università di Milano, Italy; 3) Hopital S.Vincent-de-Paul, Paris, France; 4) CSS-Mendel Institute, Rome, Italy.

Duane syndrome (MIM 126800) is an autosomal dominant disorder characterized by primary strabismus and other ocular anomalies, associated with variable deficiency of binocular sight. We have recently narrowed the Duane critical region down to a <1cM interval between markers SHGC37325 and WI4901 in a patient with Duane syndrome carrying a reciprocal translocation t(6;8)(q26;q13). To further characterize the region, we assembled a 300 kb contig spanning the translocation at band 8q13 by screening PAC and BAC libraries, and by subcloning two YACs mapping in the region. The contig was ordered using FISH and PCRs. Seven clones cover the 300 kb minimal Duane syndrome region and a single clone (co53-3) contains the breakpoint of the patient with the translocation within a completely sequenced EcoRI fragment. A combination of sequencing, database search and hybridization experiments allowed us to identify similarities in the region to a few ESTs, none of which so far related to known genes. In some cases the EST turned out to be cloning artifacts during the cDNA library manufacturing. At present, four of the mapped ESTs have been precisely positioned in the contig, two of them within cosmid co53-3. In three of these putative genes the position in the contig and the transcription direction are compatible for them to be affected by the translocation event. Two of them span the same region and are transcribed by different DNA strands. Efforts are in progress to completely characterize these genes and to positionate their exons according to the breakpoint position. Expression studies so far performed suggest for all these genes very low expression levels in the analyzed tissues.

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BAC based physical map of the distal hereditary motor neuropathy (HMN-J) and autosomal recessive inclusion body myopathy (AR-IBM) region on chromosome 9p21.1-p12. *V. Neocleous¹, S.J. Humphray², P.J. Howard², S. Hammond², M. Tsingis¹, A. Georgiou¹, A.K. Al-Qudah³, A. Mubaidin³, K. Horani³, V. Askanas⁴, K. Engel⁴, M. Dalakas⁴, L.P. Rowland⁴, M. Mirabella⁴, E. Zamba¹, T. Kyriakides¹, L.T. Middleton⁵, K. Christodoulou¹.* 1) The Cyprus Institute of Neurology & Genetics, Nicosia, Cyprus; 2) The Sanger Centre, Wellcome Trust Genome Campus, Cambridge, U.K; 3) University Hospital, Amman, Jordan or King Hussein Medical Centre, Amman, Jordan; 4) University of Southern California School of Medicine, Los Angeles, U.S.A. or NIH, Bethesda, U.S.A. or Columbia University, New York, U.S.A. or Catholic University and U.I.L.D.M., Rome, Italy; 5) Glaxo Wellcome, London, U.K.

The Jerash hereditary motor neuropathy (HMN-J) gene has recently been mapped to an approximately 0.54 cM region between microsatellite marker loci D9S1845 and D9S1791 on chromosome 9p21.1-p12. Autosomal recessive inclusion body myopathy (AR-IBM) has been mapped to chromosome 9p1-q1 and more recently refined to the 9p1 region with linkage disequilibrium of the disease mutation and a specific allele of locus D9S1791 in the Persian-Jewish population. We have developed a sequence-ready map of the region. We used a number of sequence-tagged sites (STS) from 9p21.1-p12 to screen large insert bacterial chromosome (BAC) libraries and clones were assembled into contiguous sets (contigs) by STS-content analysis and HindIII fingerprinting. Contigs were extended by the generation of additional STSs from the end sequences of bacterial clones that were used to re-screen the BAC library. A series of contig extensions were performed until the gaps were bridged and a complete contig of the region was constructed. Selected clones from the contig are currently being sequenced. The BAC contig of the region will be presented. Haplotypes of individuals from the HMN-J and AR-IBM families are currently under re-construction taking into consideration the physical order of loci and by analysis of additional loci that exist within the region. The newly established haplotypes, recombination events and refined candidate HMN-J and AR-IBM gene intervals will be presented.

Fine mapping and candidate gene screening of the deafness locus DFNA10. *S. Wayne¹, A.H. Chen¹, S. Prasad¹, K. Fukushima², C.M. Nelissen¹, K. Verhoeven³, G. Van Camp³, L. Tranebjaerg⁴, R.J.H. Smith¹.* 1) Department of Otolaryngology/Head-Neck Surg, Univ Iowa, Iowa City, IA; 2) Department of Otolaryngology, Okayama University, Okayama, Japan; 3) Department of Medical Genetics, University of Antwerp, Antwerp, Belgium; 4) University of Tromso, Tromso, Norway.

Progressive late-onset sensorineural hearing loss, or presbycusis, is a major handicap for the elderly, and is thought to be caused by a complex interaction between environmental and genetic factors. The characterization of genes associated with presbycusis may foster the development of new habilitation options. DFNA10, a previously localized locus associated with autosomal dominant, progressive, late-onset sensorineural hearing loss is a potential gene involved in presbycusis. It was previously mapped to a 17 centimorgan (cM) interval on chromosome 6q22-23.

The DFNA10 family pedigree was expanded, and family members were genotyped for polymorphic markers saturating the original DFNA10 interval. Genes and ESTs in the interval were identified using Science Genemap99. Cochlear expression of ESTs was tested by PCR amplification of the ESTs in a cochlear cDNA library. ESTs were characterized further were by 5' and 3' rapid amplification of cDNA ends (RACE) to determine the complete coding sequence. Mutation screening was accomplished using cDNA isolated from lymphoblasts from one affected family member. PCR products were sequenced in both directions and were compared to unrelated, normal hearing controls.

Key recombinant family members were identified allowing refinement of the DFNA10 interval to 1-2 cM flanked by D6S472 and D6S975. Three genes were identified in the interval, two of which (ribosomal protein S12 and connective tissue growth factor) are cochlear-expressed. No mutations were found in ribosomal protein S12. Screening of connective tissue growth factor is currently underway. Four ESTs were identified in the interval, two of which are cochlear-expressed. Further characterization of these ESTs by RACE is currently in progress.

Mutation rate at fibroblast growth factor receptor 3 (FGFR3) CpG dinucleotide 1137-1138. *S.J. Henderson¹, L. Ferguson¹, V. Macaulay², J. Loughlin¹, B. Sykes¹.* 1) Cellular Genetics, Oxford University Institute of Molecular Medicine, UK; 2) Statistics Department, Oxford University, UK.

Achondroplasia is the commonest form of dwarfism and is transmitted as an autosomal dominant trait although most cases are sporadic. A G1138A transition in the FGFR3 gene accounts for 97% of cases. New mutations show exclusive paternal origin and occur at a CpG dinucleotide. CpG dinucleotides have high mutation rates, however the G1138A transition occurs at a much higher frequency (1.38×10^{-5} per generation) than transitions at other CpG dinucleotides. The reasons for this elevated mutation rate are unknown. The G1138A mutation involves the transition of the G residue of the CpG to an A residue. Since it is the C of a CpG pair that is susceptible to mutation, it is probable that this G to A transition is the result of the substitution of the C on the non-coding strand to a T. If FGFR3 CpG dinucleotide 1137-1138 were intrinsically susceptible to a high level of mutation, it might be expected that the C residue on the coding strand (nucleotide 1137) would mutate at similar elevated rate. This would result in a C1137T transition, which is a conservative change and would not substitute the tyrosine residue. We posed the question "if the silent C1137T mutation was occurring at the same elevated rate as the G1138A achondroplasia mutation would we expect to see it in a sample of 1000 chromosomes as a common polymorphic variant?" This depends on the length of the tree that connects these chromosomes together in human history. Two models commonly used are (1) sudden expansion of humans 60000 years ago or (2) constant population with effective size of around 10000 individuals. Using model (1) we calculated that we would expect to find 33 (+/-6) mutations in 1000 chromosomes, using model (2) we would expect 4 (+/-2) mutations. However, we did not find a C1137T mutation in any of the 1000 chromosomes we screened by T-track sequencing. This suggests that the transition rate at the FGFR3 1137-1138 dinucleotide may be lower than 1.38×10^{-5} and other factors such as functional selection of sperm carrying FGFR3 mutations may be responsible for the high birth incidence of achondroplasia.

Is the pro α 2(I)collagen R618Q allele associated with specific connective tissue disorders or a rare variant? A.N. Vomund¹, S.R. Braddock², A. Cully¹, D. Chen³, M. Pepin³, P.H. Byers³, M. Godfrey⁴, C.L. Phillips^{1,2}. 1) Dept. of Biochemistry; 2) Dept. of Child Health, Univ. of Missouri-Columbia, Columbia, MO; 3) Dept. of Pathology, Univ. of Washington, Seattle, WA; 4) Human Molecular Genetics, Univ. of Nebraska Med. Ctr., Omaha, NE.

Mutations in fibrillin (FBN1) cause Marfan syndrome (MFS). Failure to identify abnormalities in FBN1 in all Marfanoid patients suggests that mutations in other genes could be involved. Phillips et al. (J Clin Invest 86:1723-8, 1990) identified a substitution (R618Q) in one pro α 2(I)collagen (COL1A2) allele of an individual with a variant of MFS. Later, another case was identified, suggesting that COL1A2 could be a MFS locus. The purpose of the present study was to determine the prevalence of the R618Q allele in normal and connective tissue patient populations. Clinical data from 316 patients referred for fibrillin immunohistochemistry due to concerns of MFS were reviewed. 123 cases had normal microfibrillar immunofluorescence (UNMC). Cultured fibroblasts from 17 of these patients, who had been ascertained because of dolichostenomelia, ectopia lentis and/or aortic dilatation or dissection, were assayed for R618Q. DNA samples from 470 control individuals were also assayed. In addition, approximately 6000 additional samples referred for evaluation of Ehlers-Danlos syndrome or osteogenesis imperfecta (1992-1999, UW) were studied at the protein level to identify the characteristic altered mobility of the α 2(I) chain and the R618Q allele was identified at the gene level. The R618Q allele was found in the original 2 MFS reported, one normal, and 12 patients with other specified connective tissue disorders. In the last group the R618Q allele did not cosegregate with the referring disorder in the 3 families in which it could be studied. The allele frequency of R618Q is approximately 0.1% in the normal population and in the population referred for evaluation of connective tissue disorders. Thus even though the arginine at position 618 is highly conserved in fibrillar collagens, the R618Q is likely a non-pathogenic variant. This variant could have a modifying role in pathogenesis of different connective tissue disorders.

ATM Haplotypes in five Hispanic populations with Ataxia-telangiectasia. *E.M. Mitui¹, S. Castellvi-Bel¹, R. Gatti¹, Collaborators²*. 1) Pathology, UCLA, Los Angeles, CA; 2) Investigators in the respective countries.

We have genotyped A-T families from five different Hispanic populations, with the purpose of finding common founder haplotypes and mutations. We have studied a total of 130 Hispanic A-T patients (56 Costa Rican, 24 American-Hispanic, 22 Brazilian, 20 Spanish and 8 Argentinean). Genomic DNA was isolated from whole blood and genotyped with four polymorphic microsatellite markers within and flanking the ATM gene, and interval spanning ~1.4 cM. The markers were: D11S1819, NS22, D11S2179, D11S1818). All alleles were compared to a standardized sample (CEPH 134702). We found 22 common haplotypes within and between different populations. One haplotype was common between Brazilian, American-Hispanic and Costa Rican populations, previously described as Costa Rican Haplotype [A], which carries the 5908 C>T mutation (Telatar *et al*, Mol. Gen. Metab. 64, 36-43, 1998). The identification of mutations corresponding to other common haplotypes is still in progress. Such haplotype analyses greatly simplify the search for mutations in populations with a high degree of inbreeding; they also trace ancestral haplotypes and result in larger extended pedigrees for genotype/phenotype comparisons.

Functional profile of *SOX10* mutations resulting in myelin deficiencies in both the central and peripheral nervous systems. *K. Inoue*¹, *E. Sock*², *M. Wegner*², *J.R. Lupski*¹. 1) Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Institut fuer Biochemie, Univ Erlangen-Nuernberg, Erlangen, Germany.

We have identified mutations in a myelin specific transcription factor, *SOX10*, in patients with myelin deficiencies in both the central and peripheral nervous systems accompanied by Waardenburg-Hirschsprung disease. These mutations include Y313X resulting in premature termination between the HMG DNA binding and transactivation domains, and 1400del12 resulting in disruption of the putative stop codon and an 82 amino acids extension. We hypothesized that these mutations, in sharp contrast with other mutations found in patients with Waardenburg-Hirschsprung disease without myelin involvement, may act as dominant negative allele at the cellular level and result in the unique phenotype of myelin disruption. To address our hypothesis, we have performed functional assays for these mutations using a tissue culture system. Each mutation was generated in a human *SOX10* cDNA expression vector. In addition, some other mutations previously reported, as well as extension clones with modifications, were introduced into the *SOX10* expression vector. These clones were cotransfected with luciferase reporter vectors into U138 human glioblastoma cells. DNA binding assays were performed using nuclear extracts from transfected COS7 cells. These studies revealed that transcriptional activities, as well as the synergistic capabilities of these *SOX10* mutants, were severely diminished. Interestingly, all clones, including mutants found in patients with no myelin involvement, competed with wild type *SOX10* function when they coexist in the assay system. The mutants with early termination all bind to DNA stronger than wild type. We further characterized the extension mutant 1400del12, and identified a 10 amino acid sequence in the tail that is critical to diminish the *SOX10* activity. We demonstrated that the mutations in *SOX10*, including a 10 amino acid extension mutation, can disrupt its transcriptional function. Further studies, however, will be required to define the mechanisms for dominant-negative mutations.

A growth hormone exon splice enhancer whose mutations perturb alternative splicing: an alternative way silent third base and other sequence variations can cause disease. *C.T. Moseley¹, J.A. Phillips III¹, M.A. Prince¹, P.E. Mullis²*. 1) Dept Pediatrics, Vanderbilt Univ, Nashville, TN; 2) Div Endocrinology, University Children's hospital, Inselspital, Bern, Switzerland.

Growth hormone deficiency (GHD) occurs in ~1/4000 births. One form of familial isolated GHD (IGHDII) is caused by dominant-negative IVS3 GH gene mutations which perturb GH mRNA splicing to produce large amounts of 17.5 kDa protein isoforms by exon 3 (E3) skipping. Normal GH splicing removes introns 1-4 to yield mRNAs containing exons 1-5 that encode 22 kDa mature GH. Alternative GH splicing uses different combinations of splice sites and can yield mRNAs encoding 22, 20, 17.5, 11.3, and 7.4 kDa isoforms. We characterized a GH exon splicing enhancer (ESE) perturbed by a natural IGHDI mutation. This mutation alters an ESE in exon3 (GAAGAA [GluGlu] GAAGGA[GluGly]) that follows the weak IVS2 3' splice site that must be used to produce 22 kDa GH. Expression constructs containing mutations of various ESE bases included GCAGAA (AlaGlu), GACGAA (AspGlu), GAGGAA (GluGlu), GAACAA (GluGln), GAAGGA (GluGly), GAAGAC (GluAsp) or GAAGAG (GluGlu). Interestingly, two (GAGGAA and GAAGAG) of these seven constructs would be predicted to be "silent" variants because they, like the natural (GAAGAA) ESE, also encode GluGlu. RT-PCR and sequence analysis of transcripts showed that all ESE mutations reduced amounts of the normal 22 by decreased use of the weak IVS2 3' splice site and increased amounts of 20 and/or 17.5 kDa mRNAs by increased use of a stronger, cryptic 3' splice site buried in E3. Our data show that the natural and all artificial mutations (including the two "silent", third base substitutions) that perturb the double encrypted GAAGAA sequence which encodes two codons and an ESE, also perturb alternative splicing. Our findings are of general interest because they show that ESE mutations can cause inherited disorders and suggest that 1) ESEs may regulate alternative transcript splicing of other genes and 2) mutations of these encrypted ESEs may be overlooked as potential causes of a variety of other genetic diseases.

Factor H mutation in familial and sporadic hemolytic uremic syndrome. *J.A. Goodship, M.R.H. Buddles, A. Richards, R.L. Donne, P. Warwicker, T.H.J. Goodship.* University of Newcastle upon Tyne, Newcastle upon Tyne, England.

In 1998, we reported linkage to chromosome 1q32 in 3 families with familial hemolytic uremic syndrome (HUS). In one of these families and an individual with sporadic HUS we found mutations in factor H (FH), the major regulator of the alternate complement pathway. Four factor H related genes have arisen by recent duplications and there is 90% identity between a 16.4kb region of FH including exons 18-20 and a 16.4kb region of FHR1 including exons 3-5. We have designed FH specific primers and identified two missense mutations in exon 18, a single base deletion in exon 19 and two missense mutations in exon 20. We have also identified gene conversion events in exon 18 and exon 20 in affected individuals. Some of these patients have decreased FH levels, presumably secondary to decreased secretion, whilst others have normal FH levels. It has been shown by deletion mutagenesis that exons 17-20 harbour a C3b binding domain and exon 20 has heparin binding activity. Disruption of these activities could be the basis of HUS in the patients with normal FH levels.

Genotype/phenotype studies in Turkish facioscapulohumeral muscular dystrophy (FSHD) families. *D. Ustek¹, P. Serdaroglu¹, A. Ozturk¹, D. Ravine², M. Krawczak², M. Upadhyaya².* 1) Medicine, University of Istanbul, Istanbul, Turkey; 2) Institute of Medical Genetics, Heath Park, Cardiff, UK.

FSHD is the third most common inherited neuromuscular disorder characterised by weakness of facial, shoulder girdle muscles. The FSHD gene has been mapped to the subtelomeric region 4q35 where it is closely linked to the polymorphic locus D4F104S1, defined by probe p13E-11. This probe detects two polymorphic EcoR1 loci, located at 4q35 and 10q26. The polymorphic EcoR1 fragments are composed almost entirely of multiple copies of the 3.3kb repeat sequence D4Z4. In most FSHD patients, deletion of a large number of these D4Z4 tandem repeats generates an EcoR1 fragment that is smaller (7-35kb) than that observed in the normal population (35-300 kb). The identification of specific Bln1 restriction sites within each of 10- derived repeat units has greatly facilitated FSHD diagnosis. An inverse relationship between the severity of clinical manifestation and the size of the shortened 4q35 EcoR1/Bln1 fragment has been demonstrated in British, Brazilian, American, Chinese and Japanese patients. We here describe our molecular findings in a cohort of 22 unrelated Turkish FSHD patients, of whom 10 were sporadic and 12 were familial. Clinical details were documented for these kindreds. High molecular weight DNA from an affected individual from each family was digested with restriction enzymes EcoR1/Bln1, fractionated on agarose gel and the Southern blots were hybridised with radio-labeled p13E-11 probe. We found suggestive evidence for a correlation between the age of onset and the size of FSHD associated p13E-11 fragment in the sporadic cases (Spearman rank correlation $r=0.54$; $p<0.2$), but not in the familial cases ($r = -0.04$; $p=0.08$). This difference between the sporadic and familial cases could be due to a higher morbidity in sporadic cases as opposed to familial cases and may reflect different genetic background. Evidence for this assertion is shown by the fact that age of onset among sporadic cases (18.3 ± 5.9 years) was significantly lower than for familial cases (27.6 ± 9.9 years); $t=2.60$, 20df, $p<0.01$. To gain a better insight of these puzzling findings, it is proposed to extend the study panel.

Clinical and molecular aspects of 25 Brazilian Friedreich's patients. *L.M.J. Albano¹, M. Zatz², C.A. Kim¹, D. Bertola¹, S.M.M. Sugayama¹, M.J. Marques-Dias³, F. Kok³, I. Ferrareto⁴, S. Rosemberg³, S. Cocozza⁵, A. Monticelli⁵.*

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We studied the clinical aspects and the frequency We studied the presence and the size of expanded GAA repeats in the first intron of X25 gene and their influence on neurologic findings, age at onset and disease progression, in 25 Brazilian patients with clinical diagnosis of Friedreich's ataxia (FA) - 19 typical and 6 atypical. Homozygous GAA expansion repeats were detected in 17 cases (68%) - all typical cases. Among typical cases, 89,5% (17/19) presented GAA homozygous expansion. In 8 patients (32%) (6 atypical and 2 typical), none expansion was observed, therefore they were not FA. In cases with GAA expansions, neurologic findings as extensor plantar responses, absence of deep tendon reflexes, postural and vibratory sense and pes cavus occurred more frequently. Electrocardiogram abnormalities were observed in 100% of GAA expansion cases against only 25% in cases without expansion. On the other hand, abnormalities in cranial nerves and in tomographic findings were detected more frequently in patients without GAA expansions. There was no genotype-phenotype correlation between the size of expanded GAA repeats and clinical aspects. We concluded that the diagnosis of FA based only in clinical findings is limited, therefore, molecular analysis is imperative to establish the diagnosis of FA.

High frequency of 4qter-10qter subtelomeric exchanges in Italian families with Facioscapulohumeral muscular dystrophy (FSHD). G. Galluzzi^{1,2}, E. Ricci^{2,3}, L. Colantoni^{1,2}, M. Rossi^{2,3}, E. Bonifazi¹, F. Mangiola², P. Tonali³, L. Felicetti¹. 1) Institute of Cell Biology, CNR, Rome, Italy; 2) Center for Neuromuscular Diseases, UILDM, Rome, Italy; 3) Institute of Neurology, Catholic University, Rome, Italy.

Sequence homology between 4qter and 10qter loci has been shown to facilitate interchromosomal exchanges resulting in the reshuffling of 4q-type BlnI-resistant and 10q-type BlnI-sensitive, KpnI repeats from one chromosome to the other. In order to verify whether the occurrence of interchromosomal exchanges could play a pathogenic role in association with 4q35 rearrangement responsible for Facioscapulohumeral muscular dystrophy (FSHD), we analyzed the segregation of BlnI-resistant and BlnI-sensitive alleles in members of 18 FSHD Italian families. Ficoll-purified leukocytes were included in agarose blocks: DNA extraction and subsequent restriction steps with EcoRI, BlnI and Tru9I were performed directly in agarose. After separation by PFGE, BlnI-resistant and BlnI-sensitive alleles were identified by using p13E-11 and KpnI cloned sequences as probes. We observed the presence of interchromosomal exchanges in 66.6% of the families studied: the exchanges were found both in affected and normal individuals and included all kinds of rearrangements (*trisomy*, *monosomy* and partial translocations). Among the 18 probands, ten (55.5%) showed the canonical 4:2 pattern of alleles while five were *trisomic* and three *monosomic*. All the *monosomic* patients carried only the small BlnI-resistant fragment related to the disease. The larger allele was a hybrid chromosome containing a mixture of BlnI-resistant and BlnI-sensitive repeats, identified only by hybridization with KpnI sequences (*spurious monosomy*). When the parental origin could be assessed, we observed that in most cases the translocated allele was inherited from the parent not transmitting the disease. Our data suggest that the basic mechanism underlying the pathogenesis of the disease is a marked instability of the KpnI subtelomeric repeats of homologous 4qter and 10qter loci, while the small 4q35 fragment causing FSHD is rarely involved in interchromosomal exchanges.

Analysis of Spinocerebellar Ataxia Types 1,3,6,7, and 8 Genes In Colombian Individuals. *C. Duran¹, M.L. Gomez¹, O.L. Pedraza³, J.C. Prieto^{1,2}*. 1) Inst de Genetica Humana, Univ Javeriana, Bogota, Colombia; 2) Hospital la Victoria. Dpto de Genetica, Bogota, Colombia; 3) Hospital San Ignacio. Dpto Neurologia, Bogota, Colombia.

The ataxias are a clinically and genetically heterogeneous group of neurodegenerative diseases that variably affect the cerebellum, brainstem and spinocerebellar tracts. Seven spinocerebellar ataxia genes (SCA1, SCA2, SCA3, SCA6, SCA7, SCA8 and SCA12) and a related dominant ataxia gene (DRPLA) have been cloned, allowing the genetic classification of these disorders. We collected clinical information and blood samples from individuals with ataxia representing twenty-three kindreds. Of the 23 kindreds, 7(31%) were classified as recessive and 16 (69%) as sporadic. All patients had gait and limb ataxia, dysarthria (90%), nystagmus (17%), pyramidal tract signs (21%), neuropathy (21%), retinal degeneration (3%), deafness (3%), movement disorder (6%), epilepsy (7%) and intellectual impairment (21%). These patients showed a wide range in the age-of-onset from 1 to 59 years and did not show genetic anticipation. MRI of the brain in affected individuals revealed cerebellar atrophy (72%) and atrophy of the brainstem (12%). We investigated the normal size range of the SCA1, SCA3, SCA6, SCA7 and SCA8 genes by genotyping of normal Colombian individuals. The ranges of the numbers of CAG repeat were: SCA1 4-13, SCA3 9-31, SCA6 7-14, SCA7 11-17 and SCA8 17-42. The frequencies of all alleles, and % heterozygosity were estimated for the Colombian mestizo population. These values were compared to values of several different ethnic groups. We analyzed the CAG expansion of SCA in affected patients and did not find SCA mutations in any patient analyzed. Variable percentages of known CAG repeat expansion have been reported in other ADCA studies, ranging from 14% in British families, to 85% in German families. In the Spanish population, SCA2 is the most common genotype (15%). The variability in these results is correlated with racial differences and dissimilar genetic backgrounds. Our preliminary results thus suggest that SCA2 ataxia or other as yet unidentified mutations are the most common in the Colombian population.

Transgenic mice carrying the human myotonic dystrophy region: a model for CTG repeat instability and its pathophysiological consequences. *H. Seznec¹, C. Savouret¹, O. Agbulut², L. Ourth¹, C. Duros¹, N. Tabti², J.C. Willer², A. Hagege³, G. Butler-Browne², C. Junien¹, G. Gourdon¹.* 1) Inserm U383, Hopital necker, Paris, France; 2) Hopital Pitie-Salpetriere, Paris, France; 3) Hopital Boucicault, Paris, France.

The molecular basis of myotonic dystrophy (DM1) is a CTG expansion located in the 3' UTR of the DMPK gene. Dramatic instability with large intergenerational increases and high levels of somatic mosaicism are observed in patients. There is now circumstantial evidence that CTG expansions affect the expression of neighboring genes and that the abnormal DMPK RNA may have detrimental effect on RNA metabolism. Transgenic mice carrying 45 kb of the human genomic DM region with the DMWD, DMPK and SIX5 genes and either 20, 55, or 320 CTG have been used to study: i) the CTG repeat instability ii) the implication of the CTG repeat expansion on neighboring genes expression iii) if the expression of a 3'UTR with expanded CTG could lead to a DM phenotype. Analyses of the CTG repeat in the 3 models (DM20, DM55, DM300) over more than 5 generations showed that the CTG repeat instability is very similar to that observed in DM patients suggesting that large human genomic sequences are required to recreate the CTG repeat instability. To define which DNA repair could be involved in the CTG repeat instability, we crossed our mice with mice knock out for different DNA repair genes. Results will be discussed. Furthermore, we are studying intergenerational instability during spermatogenesis using flow cytometry. Various phenotypic analyses or tests have been performed on the DM300 mice in muscle, eye, and heart and for behavior and insulin resistance. No abnormality has been observed in eyes and heart and behavior tests on 5 months old mice were normal. However, histological analyses in muscle showed an increase in central nuclei and a variation in the muscle fiber size. In addition, some hemizygous and homozygous mice for the transgene carrying the large 320 CTG repeat are smaller (average weight 50-60%), develop abnormal teeth after weaning and present myotonia. These results are in concordance with the RNA dominant mutation model.

SCA8 is not a major etiological factor among ataxia patients in Ontario. *J.P. Tzountzouris, D.L. Kennedy, C.R. Pereira, A. Rezaie, M. Skuterud, J. Steer, D.J. Allingham-Hawkins.* Genetics, North York General Hospital, Toronto, Ontario, Canada.

Spinocerebellar ataxia (SCA) is a genetically heterogeneous, clinically indistinguishable group of diseases characterized by ataxia, dysphagia and dysarthria with onset of symptoms usually in the third or fourth decade of life. Seven genes responsible for autosomal dominant SCA have been cloned and several others have been mapped: SCA1 (ataxin-1) at 6p22-p23; SCA2 at 12q24; SCA3/Machado-Joseph Disease at 14q32.1; SCA6 (a1A-voltage dependent calcium channel) at 19p13; SCA7 at 3p12-p13; SCA8 at 13q21; and SCA12 at 5q31-q33. In all cases, the disease appears to be caused by expansion of a polymorphic trinucleotide repeat. Previously, we have reported that, among our adult ataxia patients, SCA1 occurs in approximately 1%, SCA2 in 2%, SCA3/MJD in 7%, SCA6 in 1% and SCA7 in 3%. In addition, we have found that Freidreich's Ataxia occurs in about 7% of these patients. We have now extended our testing to include SCA8. Amongst 138 adult ataxia patients, no expanded alleles were detected. Allele sizes ranged from 15 to 31 repeats with the most common allele size being 22 repeats (37%). Thus, SCA8 does not appear to be an important etiological factor among our referral population. Further testing is underway to increase the numbers of patients tested in an attempt to establish a incidence of this form of SCA in our population. We have no plans currently to include SCA12 in our panel of tests as this form appears to be exceedingly rare, so far reported in a single family.

Functional study of a unique polyglutamine stretch in the osteoblast-specific transcription factor CBFA1. G. Zhou, B. Lee. Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

CBFA1 is one of the three known mouse orthologs of the *Drosophila* runt gene. In humans, it contains an N-terminal stretch of consecutive polyglutamine (23Q) and polyalanine (17A) repeats (Q/A domain), a DNA-binding runt domain and a C-terminal proline/serine/threonine-rich (PST) activation domain. *Cbfa1* is a non-redundant transcriptional activator of the osteoblast cell lineage; its inactivation in mice results in the absence of osteoblast and bone formation. Furthermore we and others have described mutations in *CBFA1* in patients with cleidocranial dysplasia (CCD), a dominantly inherited skeletal dysplasia marked by delayed ossification. Mutation analyses show that variable loss of function due to alterations in the polyalanine, runt and PST domains may give rise to phenotypic variability in CCD. While polyglutamine expansions have been described to underlie several neurodegenerative disorders, the function of this unique polyglutamine tract in *CBFA1* in skeletogenesis remains unknown. To this end, we generated in-frame 29Q-99Q expansions and deletions in *CBFA1* for transactivation and transgenic mice studies. Interestingly, these expansions did not have significant effect on *CBFA1* transactivation of a reporter gene while deletion of polyglutamine resulted in more than 50% reduction in its transactivation. Similar to polyglutamine disease proteins nuclear aggregates were also observed in transient transfection in COS7 cells by immunofluorescence staining with anti-*CBFA1* antibody. To determine the potential effect of polyglutamine expansion on skeletal development, a 72Q mutant *CBFA1* cDNA has been inserted downstream of a 2.3kb osteoblast-specific *Colla1* promoter and upstream of an *IRES-lacZ-pA* reporter cassette. Neonatal mice for two transgenic lines exhibited strong X-gal staining only in bone but showed no obvious developmental defects. Postnatal analysis of skeletal morphology and ossification are being performed to determine whether a dominant gain of function may be attributed to these mutations in osteoblasts, such as low bone mass. Our study will lend insight into the function of the polyglutamine stretch in *CBFA1* during skeletogenesis.

The deleterious effect of polyglutamine aggregation in cell models of Huntington's disease. *D.C. Rubinsztein¹, J. Carmichael¹, J. Rankin¹, J. Chatellier², A. Woolfson³, A. Wytenbach¹, C. Milstein³, A.R. Fersht².* 1) Medical Gen, Cambridge Univ, Cambridge Inst Medical Res, Cambridge, England; 2) Centre for Protein Engineering and Cambridge University Chemical Laboratory, MRC Centre, Hills Road, Cambridge CB2 2QH, UK; 3) MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK.

Huntington's disease (HD) is caused by expansions of >35 uninterrupted CAG repeats in exon 1 of the huntingtin gene. The CAG repeats in HD and the other 7 known diseases caused by CAG codon expansions are translated into long polyglutamine tracts, that confer a deleterious gain-of-function on the mutant proteins. Intraneuronal inclusions comprising aggregates of the relevant mutant proteins are found in the brains of patients with HD and related diseases. It is crucial to determine whether the formation of inclusions is directly pathogenic, since a number of studies have suggested that aggregates may be epiphenomena or even protective. We present two lines of evidence supporting a causal role for aggregation in polyglutamine diseases: 1) We investigated the association between aggregation and pathology with non-polyglutamine aggregates, by expressing green fluorescent protein (GFP) fused to 19-37 alanines in COS-7 cells. No aggregates were seen in cells expressing native GFP or GFP fused to 7 alanines. Aggregate-containing cells expressing GFP fused to 19-35 polyalanines show high rates of nuclear fragmentation compared to cells expressing the same constructs without aggregates, or cells expressing GFP fused to 7 alanines. This association was similar to what we observed with polyglutamine aggregates. 2) We show that fragments of the bacterial chaperone GroEL and full-length yeast Hsp104 reduce both aggregate formation and cell death in mammalian cell models of HD. In addition, a monomer comprising GroEL residues 191-345 and an artificial heptamer formed by seven copies of a GroEL minichaperone (residues 191-376) have chaperone activity in mammalian cells. Thus, we resolve another important debate by providing direct evidence that the large central cavity of GroEL is not essential for its aggregate-reducing activity in vivo.

Microarray-based profiling of gene expression changes in myotonic dystrophy (DM1): evidence for a global defect in RNA metabolism. *K.I. Virtaneva¹, P. Tamayo², X. Gao¹, J. Palatini¹, T. Wise¹, B. Udd³, T. Ashizawa⁴, F.A. Wright¹, R. Krahe¹.* 1) Div. Human Cancer Genetics, CCC, Ohio State Univ., Columbus, OH; 2) Whitehead Inst. Biomed. Res., Cambridge, MA; 3) Neurological Dept., Vasa Central Hosp., Finland; 4) Dept. of Neurology, Baylor Coll. Med. and V.A. Med. Ctr., Houston, TX.

Myotonic Dystrophy (DM1) is an autosomal dominant disorder with a pleiotropic phenotype. Its variable phenotype and anticipation have been linked to a (CTG)_n repeat expansion in the 3'-untranslated region of DM protein kinase (DMPK) gene. The expansion does not appear to affect DMPK transcription and pre-mRNA processing. However, recent studies indicate a gain-of-function for transcripts containing mutant (CUG)_n expansions, which affect RNA metabolism in cis or trans at various levels, including transcription, post-transcriptional processing and nuclear transport. We have used gene expression profiling with microarrays as an ideal system to test these hypotheses. We used the oligo-based 6,800 Human FL GeneChip array (Affymetrix, Santa Clara, CA) to globally compare expression for 6,606 non-redundant cDNAs and ESTs in skeletal muscle biopsies and cell lines of normal and DM1 individuals. Comparison of expression profiles of a pool of 10 normal skeletal muscle biopsies with six DM skeletal muscle biopsies indicated dysregulation of numerous genes involved in skeletal muscle contraction and cell cycle arrest. Similar changes were seen in vitro with primary muscle cell lines from skeletal muscle of DM1 patients. There appeared to be a block in differentiation of DM1 myoblasts into multinucleated myotubes. Moreover expression profiles of nuclear and cytoplasmic fractions, both in myoblasts and differentiated myotubes, indicated a block in nuclear transport. Together, this suggests a global trans-effect of the DM1-associated (CTG)_n expansion. We propose that the nuclear accumulation of mutant DMPK transcripts with (CUG)_n expansions causes a global defect in RNA processing in trans, which is reflected in vitro by the inability of the DM1 myoblasts to differentiate normally into multinucleated myotubes.

From molecular accomplices to pathways in the pathogenesis of HD: The roles of novel DED containing interacting proteins, HIP1 and Hippi. *R. Singaraja¹, F. Gervais², A. Hackam¹, S. Xanthoudakis², V. Houtzager², D. Rasper², S. Roy², B. Leavitt¹, C-A. Gutekunst¹, M. Hayden¹, D. Nicholson².* 1) Dept of Medical Genetics, Univ British Columbia, Vancouver, B.C; 2) Dept of Biochem, Merck Frosst Centre for Therapeutic Research, Quebec.

Huntington disease(HD)is caused by the expansion of a CAG tract in the protein huntingtin. Several huntingtin interacting proteins have been identified in an effort to elucidate the molecular mechanisms of huntingtin mediated neurotoxicity. One of the interacting proteins identified thus far, HIP1(huntingtin interacting protein1), displays a reduced association with mutant huntingtin. HIP1 contains a death effector domain (DED), and overexpression of HIP1 results in the induction of cell death in a DED dependant manner. In order to further explore this death pathway, the DED of HIP1 was used as the bait in a yeast two hybrid library screen. We isolated a novel protein, termed HIP1 protein interactor(Hippi), which contains a DED that shows striking similarity to the DED in HIP1. The DEDs of Hippi and HIP1 both contain identical sequences in their fifth helices. The presence of a charged lysine(K) residue in HIP1 and Hippi distinguish them from the DEDs of caspase-8, caspase-10 and FADD which contain a hydrophobic residue(L, V, or C) in the same location. The cellular toxicity caused by HIP1 is significantly increased in the presence of Hippi, and is dependant on the DEDs of both proteins. Caspase-8 co-immunoprecipitates with the HIP1/Hippi complex, and co-expression of dominant negative caspase-8 reduces HIP1/Hippi mediated toxicity, suggesting that caspase-8 plays an important role in this apoptotic pathway. HIP1 and Hippi are both expressed in the striatum, the region of cell death in HD. The interaction between HIP1 and Hippi increases in the presence of mutant huntingtin. Together, these data highlight a pathway for the pathogenesis of HD in which wild type huntingtin sequesters HIP1 from Hippi. In the presence of the mutation in huntingtin, HIP1 is released and binds to Hippi, initiating a caspase-8 dependant apoptotic pathway with associated increase in caspase-3 activity, resulting in neuronal cell death in HD.

L Ferritin Forms Crystalline Cataracts in Hyperferritinemia Cataract Syndrome. *D.G. Brooks^{1,3}, K. Manova-Todorova², D. Stambolian³*. 1) Medical Genetics, University of Pennsylvania, Philadelphia, PA 19104; 2) Molecular Cytology Core Facility, Memorial Sloan Kettering Cancer Center,; 3) Department of Genetics, University of Pennsylvania.

Hyperferritinemia cataract syndrome (HCS) is characterized by cataracts, hyperferritinemia and clustering of mutations in a regulatory region (iron responsive element, IRE) of the L ferritin gene. In the 5' untranslated region of the L ferritin mRNA, such mutations impair binding of iron regulatory proteins thereby disinhibiting ferritin translation. The mechanism by which the resulting ferritin protein overexpression leads to cataracts is unknown. We have identified heterozygous IRE mutations in the first 4 U.S. families with HCS. These mutations include A40G, G51C and C33T in 2 unrelated families. The fact that each of these mutations has been reported in unrelated European or Canadian HCS kindreds suggests they arose independently. One affected individual with a proven C33T IRE mutation suffered progressive glare and decreased visual acuity prompting cataract surgery. Histology of the lens aspirate revealed two types of proteinaceous deposits: Small deposits that stained as amyloid. Large crystalline-appearing deposits that diffract light, strongly suggesting ordered internal structure. By EM the latter deposits are composed of regularly-arrayed, round-shaped monomers with approximate diameter of 15 nm. Importantly, the crystals are composed of L ferritin, based on immunohistochemical analysis with mono- and polyclonal Abs. Therefore, cataracts in hyperferritinemia cataract syndrome are crystals of overexpressed L ferritin. The ability of human L ferritin to form crystals in vivo is a example of protein aggregation/insolubility. This cataractogenic mechanism confirms the long standing hypothesis that loss of lens transparency may result from large protein aggregates. Future research into the nature of amyloid deposits in HCS lens is important as this is the first report of amyloid in human lens. Ongoing electron diffraction analysis will test the hypothesis that the crystal monomer is the 24 subunit form of holoferritin. Supported by grant 1 K08 EY 00419-01 (DGB) and 5R01EY10321-06 (DS).

X-linked congenital stationary night blindness (CSNB): physical mapping and candidate analysis. *N.T. Bech-Hansen¹, R. Sparkes¹, M.J. Naylor¹, T.A. Maybaum¹, B. Koop², A.A.B. Bergen³, R.G. Weleber⁴, S.G. Jacobson⁵, J. MacGregor¹, CSNB Consortium¹.* 1) Univ Calgary, Calgary, AB., Canada; 2) University of Victoria, B.C., Canada; 3) The Netherlands Ophthalmic Research Institute, Amsterdam, The Netherlands; 4) Casey Eye Institute, Oregon Health Sciences University, Portland, OR; 5) Schei Eye Institute, Philadelphia, PA.

X-linked CSNB consists of two clinical entities, which are distinguishable by their ERG abnormalities. Mutations in CACNA1F, a calcium channel alpha-1-subunit gene, is responsible for incomplete CSNB (Nature Genet. 19:264-267, 1998). Complete CSNB is a defect of the ON-pathway involving both rod and cone signals. The gene for complete CSNB (CSNB1) maps to an adjacent but distinct location from CACNA1F on the X chromosome, but is still unknown. Families with complete CSNB were analyzed to refine the position of the CSNB1 gene. New genetic markers were established and used to narrow the minimal region of the CSNB1 locus within Xp11.4. A 1.2 Mb physical map of the CSNB1 region was constructed of BACs and PACs, and a transcript map of this region was established from large-scale genomic DNA sequence and BLAST analysis. Candidate genes were identified for mutations analysis. In particular, the genomic organization of the CASK gene, whose expression includes the retina, was found to span in excess of 200 kb within the CSNB1 minimal region. So far five candidate genes, including CASK and Fat Facet, have been excluded as the CSNB1 gene. Additional genes, which are expressed in the retina and map to the CSNB1 minimal region, are being analyzed for mutations. Conclusions: The map position of the CSNB1 gene responsible for complete CSNB has been refined to a 1.2 Mb region of Xp11.4. Patients with complete CSNB showed no mutations in the CASK and FAT FACET genes, excluding them as the CSNB1 gene. Additional candidate genes from the CSNB1 region transcript map are being analyzed for mutations and is expected to identify the gene in Xp11.4 that causes the X-linked retinal disorder complete CSNB. MRC and RP Research Foundation (Canada), AHFMR, and Roy Allen Endowment.

An A(-71)C substitution in the promoter of the green visual pigment gene is associated with deutan color vision defects. *T. Hayashi*¹, *H. Ueyama*², *S. Oda*³, *Y. Tanaka*², *S. Yamade*³, *S.S. Deeb*¹. 1) Medicine and Genetics, University of Washington, Seattle, WA; 2) Second Biochemistry, Shiga University of Medical Science, Shiga, Japan; 3) Ophthalmology, Shiga University of Medical Science, Shiga, Japan.

The red and green visual pigment genes are arranged in a head-to-tail tandem array on chromosome Xq28. The great majority of deutan color vision defects among Caucasians are caused either by deletion of the green pigment gene or by its conversion to a green-red hybrid gene. We studied the red/green gene arrays of 116 Japanese males (from the Shiga and Aichi regions of Japan) with deutan color vision defects. We found that 12 of these deutan males have one normal red and one normal green pigment genes but no green-red hybrid genes. Promoter analysis revealed an A to C substitution at position -71 of the green pigment gene in 9 of these 12 subjects. This promoter substitution was not found in 52 color-vision normal males from the same region of Japan whose arrays are comprised of one normal red and one normal green pigment genes. However, this substitution was carried, along with the normal allele, by 33 of 67 males with normal color vision whose arrays contain a normal red and more than one green pigment gene. Recently, we found that only the first two genes of the array are expressed in the retina and influence color vision (Hayashi et al, *Nature Genet* 22:90, 1999). Therefore, the green pigment gene with the A-71C substitution in the 33 color vision normal males most likely occupies a more distal position in the array and, therefore, does not influence color vision. The A-71C substitution was not found among Caucasians of normal or defective color vision. In conclusion, the A-71C substitution in the green pigment gene promoter may be a novel cause of deutan color vision defects. Results of the impact of this substitution on promoter activity *in vitro* will be presented.

A novel MYOC/TIGR gene mutation (Arg158Gln) in Japanese patients with open angle glaucoma. *F. Mabuchi¹, Z. Yamagata¹, K. Kashiwagi², K. Ishijima³, S. Tang¹, H. Iijima², T. Tsukahara².* 1) Dept of Health Sci, Yamanashi Medical Univ, Yamanashi, Japan; 2) Dept of Ophthalmology, Yamanashi Medical Univ, Yamanashi, Japan; 3) Dept of Ophthalmology, Uenohara City Hospital, Yamanashi, Japan.

We report a novel mutation in the myocilin/trabecular meshwork-inducible glucocorticoid response protein (MYOC/TIGR) gene in 2 Japanese patients with open angle glaucoma (OAG). Single-strand conformation polymorphism (SSCP) analysis and subsequent sequence analysis were performed for the MYOC/TIGR gene in 220 OAG patients and 89 reference patients. A novel Arg158Gln mutation in the first exon of the MYOC/TIGR gene was found in 2 unrelated patients out of the 220 OAG patients. The mutation was found in none of the reference patients. The first patient, a 43-year-old man, with no family history of glaucoma, showed high intraocular pressure (IOP) in both eyes at the age of 38. Maximum IOPs were 70mmHg and 36mmHg in his right and left eyes respectively. No visual field defects have been detected. The second case was a 74-year-old woman; she had no family history of glaucoma and had been followed for cataract and normal tension glaucoma (NTG) in both eyes. Maximum IOP was 16mmHg in each eye. The right eye showed typical glaucomatous visual field defects, while the left field was normal. A novel mutation, Arg158Gln, in the first exon of the MYOC/TIGR gene may be responsible for a subset of OAG showing various phenotypes including both ocular hypertension and NTG in the Japanese population.

Phenotypic heterogeneity of *CYP1B1* suggested by mutations in a patient with Peters' anomaly. A.L. Vincent^{1,2}, G.D. Billingsley², M.K. Priston², D. Williams-Lyn², J. Sutherland¹, T. Glaser³, E. Oliver³, A.V. Levin¹, E. Héon^{1,2}. 1) Dept Ophthalmology, The Hospital for Sick Children, University of Toronto; 2) Vision Science Research Program, UHN, Toronto, ON, CANADA; 3) Depts of Internal Medicine and Human Genetics, University of Michigan Medical Center, Ann Arbor, MI, USA.

Both Peters' anomaly and primary congenital glaucoma are developmental anomalies affecting the anterior segment of the eye. Congenital glaucoma is autosomal recessive and genetically heterogeneous. *CYP1B1* is proposed to be the predominant gene associated with primary congenital glaucoma in several published series. Peters' anomaly consists of corneal leucoma (opacity), defects in the posterior layers of the cornea, and iridocorneal and/or keratolenticular strands bilateral in 80%. Over 50% of individuals develop glaucoma in childhood. Autosomal recessive inheritance and sporadic cases are most frequently documented. Numerous etiologies have been proposed including mutations in 2 important eye development genes; *PAX6*, and *RIEGL/PITX2*, yet this accounts for only a small fraction of cases. Patients with congenital glaucoma were screened for mutations in *CYP1B1*. Mutational analysis of one patient revealed a compound heterozygous mutation for 2 previously unpublished sequence changes: a missense mutation in exon 2, 3976G→A, causing a premature stop (Trp57STOP), predicted to truncate the functionally essential heme binding site; and 3807T→C, (Met1Thr). This patient had bilateral Peters' anomaly in addition to the congenital glaucoma. He presented at birth with bilateral cloudy corneas and epiphora. EUA at 3 weeks of age showed central corneal opacities, raised intraocular pressures and iridocorneal adhesions. Left corneal decompensation at 3 months of age resulted in a penetrating keratoplasty. Histology of the corneal button showed changes resembling Peters' anomaly. There are no other affected family members. Mutational analysis of 2 candidate genes associated with Peters' anomaly, *RIEGL* and *PAX6*, failed to document any pathogenic sequence change. This case suggests congenital glaucoma and Peters' anomaly may share a common molecular background.

Genome-wide search for a genetic modifier locus in a Saudi Arabian population segregating Primary Congenital Glaucoma with incomplete penetrance. *D.W. Stockton¹, R.A. Lewis¹, K. Tomey², D.K. Dueker², M. Jabak², W.F. Astle², J.R. Lupski¹, B.A. Bejjani¹.* 1) Baylor Col Medicine, Houston, TX; 2) King Khaled Eye Specialist Hosp, Saudi Arabia.

Purpose: Primary Congenital Glaucoma (PCG) is an autosomal recessive disorder associated with unknown developmental defect(s) in the anterior chamber of the eye. PCG has traditionally been considered completely penetrant. There is, however, evidence of decreased penetrance in the Saudi Arabian population. The purpose of this study is to confirm nonpenetrance in the Saudi population and identify the molecular basis of the decreased penetrance. **Methods:** 62 Saudi PCG families were studied. Mutations and intragenic SNPs were analyzed from direct sequencing or restriction endonuclease digestion of the *CYP11B1* coding exons in all individuals. Three candidate genes for the modifier were investigated using linkage analysis and/or Southern blotting. 19 families that demonstrated nonpenetrance were used for a genome wide screen. **Results:** Eight distinct mutations were identified: the most common account for 91% of the Saudi PCG chromosomes; five additional homozygous mutations were detected, each in a single family. Affected individuals from five families had no *CYP11B1* coding mutations and each family had a unique SNP profile. Decreased penetrance was confirmed in 22 of the 62 families. Linkage and Southern analyses excluded three candidate modifier loci. Genotypes were analyzed from 303 microsatellite markers as an exclusion screen and identified several regions for the modifier locus. **Conclusions:** These data demonstrate decreased penetrance of the PCG phenotype in the Saudi population, because 40 apparently unaffected individuals in 22 families have mutations and haplotypes identical to their affected siblings. Analysis of these 22 kindreds is compatible with the presence of a dominant modifier locus that is not linked genetically to *CYP11B1*. The study of genetic isolates and inbred populations represents a strategy to map Mendelian traits rapidly and to dissect complex human disorders. The cloning of a modifier gene should substantively impact our understanding of the pathogenesis of congenital glaucoma.

Extensive screening of four disease causing genes and several candidate genes in 130 families affected with Leber congenital amaurosis. *I. Perrault¹, J.M. Rozet¹, S. Gerber¹, D. Ducroq¹, I. Ghazi², C. Leowski³, E. Souied¹, J.L. Dufier², A. Munnich¹, J. Kaplan¹.* 1) INSERM U393 - Dpt de Génétique, Hôpital des Enfants Malades, Paris, France; 2) Service d'Ophtalmologie, Hôpital Necker, Paris, France; 3) Institut National des Jeunes Aveugles, Paris, France.

Leber congenital amaurosis LCA, is the most early and severe form of retinal dystrophies. Both clinical and genetic heterogeneity have long been suspected. We report here the genetic study of a large panel of 165 families affected with LCA, originating from various countries across the world. The three already identified causing genes, *retGC1*, *RPE65* and *CRX* have been screened as well as a newly identified causing gene, *AIPL1* and some attractive candidate genes. So far, 130/165 families have been screened for mutations. Twenty-two different mutations of the *retGC1* gene have been identified in 24 unrelated families (18.5%) as well as 12 different mutations of the *RPE65* gene in 9 unrelated families (7%), 2 different mutations of *CRX* in 2 families (1.5%) and finally, 4 different mutations of the *AIPL1* gene in 5 among 78 families (6.5%). Taken together, these four genes account for 33.5% of the LCA families of our panel. In addition, we report the results of the screening of some attractive candidate genes by virtue of their function. The genetic heterogeneity of LCA no more needs to be demonstrated. We speculate that a major gene apart, at least ten genes will account for Leber's congenital amaurosis involving completely different physiological pathways.

Molecular studies of AIPL1, a gene causing Leber congenital amaurosis. *D.L. Tirpak¹, M.M. Sohoeki¹, C.M. Craft², S.P. Daiger^{1,3}.* 1) Human Genetics Center, University of Texas-Houston; 2) Doheny Eye Institute & Keck School of Medicine of the Univ. Southern Calif., Los Angeles, CA; 3) Dept. of Ophthalmology and Visual Science, The University of Texas-Houston.

Leber congenital amaurosis (LCA) is the most severe inherited retinal disease and is characterized by significant loss of vision or total blindness at birth. Our laboratory recently identified a novel LCA disease-causing gene, aryl-hydrocarbon interacting protein-like 1 (AIPL1). AIPL1 encodes a 384 amino acid protein, contains three tetratricopeptide repeat (TPR) motifs, and shows similarity to the FK506 binding proteins. Collectively, these characteristics indicate that AIPL1 may function as a chaperone or nuclear transport protein. Our goal is to define the biological role of AIPL1. To this end, we used a yeast two-hybrid assay to identify AIPL1 interacting proteins. Screening of a bovine retinal cDNA library identified 33 clones containing potential AIPL1 interacting proteins. To date, follow up studies with seven of these clones has identified two putative AIPL1 interacting proteins. *In silico* analysis to identify human orthologs revealed that one clone is homologous to genomic sequence on chromosome 15p while the other maps to chromosome 5 and is homologous to a gene involved in glucose metabolism. Ongoing studies with the remaining 26 clones are being performed and *in vitro* binding assays will be conducted with all putative AIPL1 interacting proteins to confirm the yeast two-hybrid data. The identification of proteins that bind to and interact with AIPL1 will foster a more holistic understanding of its functional role in the retina, as well as in the LCA disease process. Moreover, by performing mutation screens on AIPL1 interacting proteins identified through the yeast two-hybrid system, we may be able to discover novel genes associated with retinal degeneration and modifying factors responsible for the genetic heterogeneity observed with retinopathies such as LCA. Supported by grants from the Hermann Eye Fund, The Foundation Fighting Blindness, and NIH grant EY07142.

Analysis of MYOC/TIGR gene mutations in Japanese patients with normal tension glaucoma. Z. Yamagata¹, F. Mabuchi¹, K. Kashiwagi², S. Tang¹, H. Iijima², S. Tsukahara². 1) Dept of Health Sci, Yamanashi Medical Univ, Yamanashi, Japan; 2) Dept of Ophthalmology, Yamanashi Medical Univ, Yamanashi, Japan.

We have assessed the types and frequency of myocilin/trabecular meshwork-inducible glucocorticoid response protein (MYOC/TIGR) gene mutations in Japanese patients with normal tension glaucoma (NTG). Peripheral blood was collected from 112 primary open angle glaucoma (POAG) patients, 108 NTG patients and 89 reference patients. Genomic DNA was purified from the blood samples. Single-strand confirmation polymorphism (SSCP) analysis and subsequent sequence analysis were performed for genotyping the MYOC/TIGR gene. Eight different MYOC/TIGR gene sequence variations were identified. These included 1-83 from G to A in the promotor (10 POAG, 3 NTG), Arg46Stop (1 NTG), Arg76Lys (10 POAG, 3 NTG), Thr123Thr (1 POAG), Arg158Gln (1 POAG, 1 NTG), Asp208Glu (3 POAG, 2 NTG), Ala488Ala (1 POAG), 1515+20 from G to A in the 3' untranslated region (1 POAG). Of the 8 variations, 2 (Arg46Stop and Arg158Gln) were judged to be probable disease-causing mutations. The number of patients found to harbor such mutations was 1/112 (0.9%) for POAG and 2/108 (1.9%) for NTG. The remaining 6 were regarded as polymorphism since they were found in reference patients. MYOC/TIGR gene mutations occur in Japanese NTG patients at a frequency similar to that of Japanese POAG patients. The pathogenesis of glaucoma associated with MYOC/TIGR gene mutations might be more complex than previously anticipated, and other pathogenesis unrelated to high intraocular pressure may be considered.

Identification and characterization of a gene encoding transport-like RPGR-interacting proteins (RPGRIPs) and analysis of its involvement in retinal dystrophies. R. Roepman¹, N. Bernoud-Hubac², C. Vink¹, H.H. Ropers^{1,3}, W. Berger³, P. Ferreira², F. Cremers¹. 1) Dept. of Human Genetics, University Medical Center St. Radboud, Nijmegen, The Netherlands; 2) Dept. of Pharmacology, Medical College of Wisconsin, Milwaukee, WI; 3) Max-Planck Institute of Molecular Genetics, Berlin, Germany.

Mutations in the Retinitis Pigmentosa GTPase Regulator (*RPGR*) gene cause X-linked retinitis pigmentosa type 3 (RP3), a severe and progressive retinal dystrophy leading eventually to complete blindness. The *RPGR* gene is ubiquitously expressed, though retina-specific mRNA splice variants have been reported. Yet, mutations in the *RPGR* gene lead to a retina-restricted phenotype. To date, all RP3 missense mutations that have been identified are located in the RCC1-homologous domain (RHD) of *RPGR*.

Using a yeast two-hybrid screen, we have identified several alternative-spliced gene products, which interact specifically with the RHD of *RPGR* *in vivo* and *in vitro*. Some of these isoforms are retina-specifically expressed. These novel proteins, named RPGR-interacting proteins (RPGRIPs), contain a C-terminal RPGR-interacting domain and stretches of variable coiled-coil domains homologous to proteins involved in vesicular trafficking. The interaction between *RPGR* and RPGRIPs was impaired *in vivo* by RP3-associated mutations in *RPGR*. Moreover, *RPGR* and RPGRIPs colocalize in the outer segment of rod photoreceptors. These results are in full agreement with the RP phenotype observed in RP3 patients, provide a clue for the retina-specific pathogenesis in RP3 and hint towards the involvement of *RPGR* and RPGRIPs in mediating transport-associated processes.

The *RPGRIP* gene was located in a genomic interval of 70 kb on 14q11, which makes it a strong candidate gene for RP16. It contains 25 exons and an open reading frame of 3774 bp. Mutation analysis in a large cohort of retinal dystrophy families is underway.

Characterization of mutations in families affected with Corneal dystrophy. *N. Udar, P. Vyas, S. Sheikhavandi, S. Yelchits, R. Garcia, H. Nguyen, K. Small.* Jules Stein Eye Institute, UCLA Sch Medicine, Los Angeles, CA.

A group of four corneal dystrophies are characterized by bilaterally symmetrical disorder with progressive accumulation of corneal deposits that begin to appear during the 1st or 2nd decade of life. With time these opacities cause serious visual handicaps, often requiring phototherapeutic keratectomy or corneal transplantation. These are inherited in an autosomal dominant pattern. A single gene BIGH3 was identified accounting for all four disorders - Granular, Lattice type I, Adeline and Reis Buckler. Studies have been done with different ethnic population to study the hot spot nature of these mutations. We have studied 2 caucasian families with Granular corneal dystrophy (CDGG1) and Reis Buckler (CDRB). SSCP analysis was carried out on 47 members of a Reis Buckler family with 10 affected and 21 unaffected and 80 unaffected for a Granular corneal dystrophy family. Sequence analysis revealed a R555Q mutation for the CDRB family and R555W mutation for the CDGG1 family. We carried out haplotype analysis on our individuals and found them to be different from the published ones. These results support the hot spot theory instead of the common ancestor hypothesis.

MECP2 MUTATIONS IN ITALIAN PATIENTS WITH RETT SYNDROME. *F.L. Conforti¹, R. Mazzei¹, A. Magariello¹, A. Fiumara², R. Barone², AM. Patitucci¹, AL. Gabriele¹, M. Muglia¹.* 1) Institute of Experimental Medicine and Biotechnology, National Research Council, Mangone, Cosenza, Italy; 2) Department of Pediatrics, University of Catania, Catania, Italy.

RETT syndrome is a progressive X-linked dominant disorder affecting almost exclusively females with an incidence of 1 in 10.000-15.000. Patients with classic RTT are characterised by loss of acquired skills after a period of normal development in infant girls. Familial studies showed that Rett syndrome is caused by mutations in a gene located in the Xq28 region. Recently mutations have been identified in the MECP2 gene, encoding an X-linked methyl CpG binding protein 2, a 486 aa protein which contains two domains, the methyl CpG binding domain (MBD), and the transcriptional repression domain (TRD). MECP2 protein selectively binds two methylated CpG dinucleotides in the genome and mediates transcription repression through interaction with histon deacetylase and the corepressor SIN3A. Molecular investigation of the coding region of the MECP2 gene in 12 Rett syndrome patients from Sicily (Southern Italy), by direct sequencing, has allowed us to identify de novo mutations in all of out four patients. Two of the mutations led to premature truncation of the protein, the remaining were missense mutations. All recurrent nucleotide substitutions found in our cases were C to T transition in CpG dinucleotides, reinforcing the proposed CpG sites mechanism of hypermutability at CpG sites. In conclusion, our data provide additional support for the MECP2 gene being involved in the pathogenesis of Rett syndrome.

Exclusion of MTMR1 as a Frequent Cause of X-Linked Myotubular Myopathy. *L.M. Copley¹, W.D Zhao¹, K. Kopacz¹, P. Kioschis², S. Taudien³, M. Platzner³, A. Poustka², G.E. Herman¹.* 1) Pediatrics, Children's Research Institute, Columbus, OH; 2) German Cancer Research Center, Division of Molecular Genome Analysis, Heidelberg, Germany; 3) Institute of Molecular Biotechnology, Genome Analysis, Jena, Germany.

X-linked myotubular myopathy (MTM, MIM# 310400) is a rare neuromuscular disease presenting at birth with hypotonia and characteristic facies. In 1996, an MTM1 gene encoding a predicted protein called myotubularin, was isolated. To date, mutations in more than one hundred MTM1 patients worldwide have been characterized. However, no mutation within this gene has been found in approximately 20% of MTM patients, including several males with an X-linked pattern of inheritance. MTMR1, a gene highly homologous to MTM1, is located 50KB telomeric on the X Chromosome. We have now screened all 16 exons of MTMR1 in genomic DNA from 14 MTM patients, including two with X linked pedigrees and two with affected male siblings. A single potential mutation, (G2R), was identified in a male proband from one of the X-linked families. These results suggest that mutations in MTMR1 are not a frequent cause of MTM in males for whom no mutation was found in MTM1. Further functional studies are needed to confirm if the change found in the single patient described is significant.

Mutations of a cytosine tract in exon 10 of *NEMO* (*IKK-g*) cause atypical forms of Incontinentia pigmenti. *D.L. Nelson*¹, *G. Courtois*², *A. Smahi*³, *H. Woffendin*⁴, *T. Esposito*⁵, *A. Ciccodicola*⁵, *R.A. Lewis*¹, *M. D'Urso*⁵, *S. Kenwrick*⁴, *A. Munnich*³, *A. Israël*², *S. Aradhya*¹. 1) Molecular & Human Genetics, Baylor College Medicine, Houston, TX; 2) Institut Pasteur, Paris, France; 3) Genetics, Hopital Necker-Enfants Malades, Paris, France; 4) Wellcome Trust Centre and University of Cambridge, Cambridge, UK; 5) Int'l Inst of Genetics and Biophysics, Naples, Italy.

Familial Incontinentia pigmenti (IP) is an X-linked dominant and male-lethal disorder that affects neural crest derived cells. We recently described multiple IP patients with loss of function mutations in NEMO, an activator of the NF- κ B pathway. The absence of NEMO leads to uncontrolled apoptosis, thereby causing the characteristic skewing of X-inactivation in females and lethality in males. Although most mutations of NEMO cause typical IP, we now describe families with unusual phenotypes due to mutations in exon 10. Remarkably, these families include affected male offspring that survived to term, and also exhibited signs not commonly associated with IP, including immune dysfunction and hematopoietic disturbance. We report three different mutations at the same location within a cytosine tract in exon 10, which most likely occurred due to misalignment during recombination. However, only some exon 10 mutations allow survival of males, presumably because these mutations are "mild". In support of this hypothesis, females with mild mutations demonstrate random or slight skewing of X-inactivation, a finding contrary to typical IP. Moreover, analysis of cell lines transfected with mutant forms of NEMO indicates that mild mutations only partially alter NF- κ B activation. Although counter to accepted opinion, these data emphasize that males can also suffer from IP. Our results also urge a reevaluation of the diagnostic criteria since many male IP patients may have been misdiagnosed due to previous misconceptions about lethality or the presence of medical problems not usually associated with IP. Lastly, the involvement of the immune and hematopoietic systems in IP males provides a unique opportunity to further investigate the role of the NF- κ B pathway in these systems.

Refinement of a nonsyndromic mental retardation locus in Xq24. *K.E. Miller¹, F.R. Pastrana¹, S. Ladd¹, M.C. Phelan^{1, 2}, R.G. Gregg³, C.E. Schwartz¹, R.E. Stevenson¹, A.K. Srivastava¹.* 1) Center for Molecular Studies, J.C. Self Res. Inst. of Hum. Genet., Greenwood Genetic Center, Greenwood, SC; 2) Present address: Genetics Diagnostic Lab., T.C. Thomson Childrens Hospital, Chattanooga, TN; 3) Dept. of Biochem. and Mol. Biol., University of Louisville, Louisville, KY.

Mental retardation is a common condition affecting 2-3% of the human population. X-linked forms of mental retardation (XLMR) have been noted as the most common cause of inherited MR in males. At present, more than 75 nonsyndromic (MRX) pedigrees have been mapped to a variety of loci on the X chromosome with broad intervals of assignment that can be grouped in 10-12 non-overlapping regions. To date at least six MRX genes, FMR2, GDI1, OPHN1, PAK3, IL1RAPL and TM4SF2 have been cloned. Linkage of several MRX pedigrees (MRX23, MRX30, MRX35, and MRX47) and two other more distinctive neurological syndromes (EFMR and X-linked nonprogressive cerebellar hypoplasia) to the large genetic interval, Xq22-q24 suggests the presence of several MR genes in this region. So far only the PAK3 gene from this interval has been found to be mutated in MRX30 but not in other linked families. To clone additional MR genes from the Xq23-q24 region, we have characterized a balanced translocation, t(X;7)(q24;q22.3), in a 36 year old female patient with an IQ of 44. Our preliminary FISH studies with several YACs/BACs and cosmid clones containing anchored markers placed the breakpoint between DXS1220 and DXS424. Additionally, we have also analyzed the MRX23 family whose location (DXS1220-DXS424) overlaps with the critical region defined by the X;7 translocation. We have analyzed a novel gene, symporter (GLYT1-like), from this interval as a potential candidate gene. FISH analysis mapped this gene telomeric to the breakpoint. We have identified a silent gene alteration (331T/A; E29E) of the symporter gene in MRX23. Haplotype analysis of the single nucleotide variation (331T/A) in carrier females and an affected male with known recombination in MRX23 further narrowed the MR gene location between DXS1220 and symporter and suggests the likely involvement of a single MR gene in both MRX23 and in the female MR patient with the X;7 translocation.

Novel Deletion of the RPGR gene in a Chinese Family with X-linked retinitis Pigmentosa. Kanxin. Zhao¹, Lejin. Wang¹, Li. Wang¹, Liming. Wang¹, Yu. Zhu¹, Qingsheng. Zhang¹, Shihong. Xiong¹, Yun. Cui¹, Weiyang. Chen¹, Qing. Wang^{1, 2}. 1) Laboratory of Molecular Genetics, The Tianjin Eye Hospital and The Tianjin Medical University, Tianjin, P. R. China; 2) Center for Molecular Genetics, ND4-38, The Cleveland Clinic Foundation, Cleveland, OH 44195.

Retinitis pigmentosa (RP) is a common and inherited retinal dystrophy characterized by photoreceptor cell degeneration, night blindness, a gradual loss of peripheral visual fields, and eventually loss of central vision. RP affects 1 in 4000 people and is responsible for visual handicap of 1.5 million individuals worldwide. X-linked retinitis pigmentosa is a severe form of RP. Two X-linked RP genes, RPGR (RP3, retinitis pigmentosa GTPase regulator) and RP2, have been identified by positional cloning. We characterized a Chinese family with X-linked RP. Using linkage analysis with markers covering the entire X chromosome, we established that the RP-causing gene in the Chinese RP family is the RPGR gene. Although the RPGR gene and the RP2 gene are close to each other on chromosome X, we excluded RP2 as the disease-causing gene in the family. Using single-strand conformation polymorphism (SSCP) and DNA sequence analyses, we have now identified the disease-causing mutation in the Chinese family. The RP-causing mutation is a 28-bp deletion in exon 7 of RPGR. This deletion results in an in-frame stop codon that eliminates the C-terminal half of the RPGR protein. The 28-bp deletion described here is the first RPGR mutation reported in the Chinese population. Four females in kindred RP002 are the carriers of the 28-bp deletion of RPGR. The carrier females in the family are affected with myopia and mild symptoms of RP, consistent with that the 28-bp deletion is a severe mutation in RPGR. (This study was supported by the China National Natural Science Foundation).

MECP2 truncating mutations cause histone H4 hyperacetylation in Rett syndrome. *M. Wan¹, K. Zhao², S.S. Lee¹, H.R. Song³, N.C. Schanen³, U. Francke¹.* 1) Department of Genetics/HHMI, Stanford University School of Medicine, Stanford, CA; 2) Laboratory of Molecular Immunology, NHLBI, NIH, Bethesda, MD; 3) Department of Human Genetics, UCLA School of Medicine, Los Angeles, CA.

Rett syndrome (RTT) is a mostly sporadic neurodevelopmental disorder characterized by developmental regression with loss of speech and of purposeful hand use, microcephaly, and seizures. It affects approximately 1 in 15,000 live born females. RTT is caused by mutations in the MECP2 gene, which is located in Xq28 and subject to X inactivation. MECP2 encodes a methyl-CpG-binding protein, that binds specifically to methylated cytosine through its methyl binding domain (MBD), and recruits a transcriptional silencing complex through its transcriptional repression domain (TRD), resulting in histone deacetylation and chromatin condensation. To study the biochemical effects of two common truncating mutations (R168X and 803delG) found in RTT patients, we examined MeCP2 expression and global histone acetylation levels in clonal cell cultures from a RTT patient with the mutant R168X allele on the active X chromosome, as well as in cells from a male carrying a RTT mutation (803delG) (Wan et al. AJHG 65:1520-1529, 1999). Both mutant alleles generated stable RNA transcripts, but no intact MECP2 protein was detected in either type of cell with an antibody against the C-terminal region of MeCP2. As measured by quantitative Western blots of whole cell lysates, histone H4 but not H3, was hyperacetylated. To examine which specific lysine residue is hyperacetylated, we performed Western blots using antibodies against acetyl-histone H4 at lysine 5, 8, 12 and 16. H4 hyperacetylation was only found at lysine 16 in cells expressing MECP2 mutant alleles. As predicted from the known MeCP2 function, the expression of endogenous truncating MECP2 alleles does indeed cause global histone H4 hyperacetylation. This may result in MeCP2 target gene overexpression. Therefore, H4 hyperacetylation at lysine 16 may play an important role in pathogenesis of RTT.

An amino acid deletion in the human connexin 31 gene (GJB3) is associated with sensorineural deafness and hereditary sensory neuropathy. *N. Lopez-Bigas¹, M. Olive², R. Rabionet¹, O. Bravo², I. Banchs¹, V. Volpini¹, I. Ferrer², M.L. Arbones¹, X. Estivill¹.* 1) Medical and Molecular Genetics, Cancer Research Institut, Barcelona, Spain; 2) Hospital Bellvitge, CSUB, Barcelona, Spain.

Deafness involves a high number of genes and environmental factors. Four genes causing hearing impairment (GJB1, GJB2, GJB3 and GJB6) encode connexin proteins. Mutations in GJB3 (Cx31) cause autosomal recessive and autosomal dominant hearing impairment, and erythrokeratoderma variabilis. Autosomal dominant deafness due to GJB3 mutations shows late onset and partial penetrance, while autosomal recessive deafness is associated with early onset. GJB1 (Cx32) is mutated in patients that have peripheral neuropathy, with hearing impairment being associated in some cases. We report here a mutation in the GJB3 gene in a family affected of peripheral neuropathy and sensorineural deafness. The mutation is a deletion of an asparagine at amino acid position 66 (66delD). A wide range of disease severity was detected in the affected members for both the hearing impairment and the peripheral neuropathy (from cases with only electrophysiology alterations to others with chronic skin ulcers and osteomyelitis leading to feet amputations in two patients). Mutation 66delD affects a residue highly conserved across species and across different connexins and has not been found in 400 normal chromosomes. The same mutation has been detected in the GJB1 gene in patients affected of Charcot-Marie-Tooth (Haite et al, 1998) and the same amino acid has been also found mutated (D66H) in GJB2 (Cx26) in families affected of Vohwinkels syndrome (Maestrini et al, 1999). The identification of mutations causing disease in the residue 66 in the three connexin genes (Cx26, Cx31 and Cx32) suggests that this residue is important to the function of the connexin31 gene. The wide spectrum of phenotypes and disease severity detected for GJB3 mutations (skin and the hearing system) parallels that detected for GJB2, also indicating that some mutations have a variable penetrance. The involvement of the peripheral neuron system in some GJB3 mutations may indicate that this gene may cause deafness through a different mechanism than GJB2.

Ancient Mutations in the FMF Gene: Carrier Frequency in Different Ethnic Groups in Israel and Genotype-Phenotype Correlation. *R. Gershoni-Baruch*^{1, 2}, *M. Shinawi*¹, *L. Kasinetz*¹, *R. Brick*^{1,2}. 1) Institute of Human Genetics, Rambam Medical Ctr, Haifa, Israel; 2) Bruce Rappaport Faculty of Medicine, Technion - Institute of Technology, Haifa, Israel.

Five mutations (M680I, M694V, M694I, V726A, E148Q) account for the majority of FMF chromosomes. They probably originated in Israel some 2,000 years ago and are widely, yet, differentially distributed in the various ethnic groups inhabiting the middle east. We determined their frequency in 1200 healthy individuals of Jewish (Iraqi, Moroccan and Ashkenazi) and Islamic descent. We also analyzed 300 FMF patients and correlated genotypes with disease severity. Our results show that mutations V726A and E148Q shared by Arabs, Iraqi Jews and Ashkenazim contribute to a mild, rather non-penetrant phenotype. M680I and M694I are confined to Arabs and are associated together with the M694V mutation with a severe phenotype. Mutation M694V occurs in all, other than Ashkenazi Jews. Mutation V726A occurs in all, other than Moroccan Jews. Arabs harbor all five founder mutations. Although FMF is noted among Moroccan Jews, the prevalence of FMF carrier chromosomes is higher among Iraqi and Ashkenazi Jews. Ashkenazim have drifted from the Iraqi genetic pool about 1500 years ago carrying with them the V726A mutation of which Moroccan Jews remain exempt. The question, why many different FMF carrier chromosomes have originated and propagated in the middle east remains open.

Study of methylenetetrahydrofolate reductase gene mutation as a maternal risk factor of Down syndrome in Iran. *S.A. Aleyasin, F. Jahanshad, S. Mohammad Ganji, N. Atabak.* Medical Genetic group, National research center for genetic engineering and Biotechnology, Tehran, IRAN.

Trisomy 21 is the clinically most frequent autosomal aneuploidy, observing in approximately 1 in 700 newborns. It is the most common causes of sever mental retardation. Diagnosis of this disorder is usually performed by conventional cytogenetic analysis of blood and amniotic or chorionic fetal cells. However, this technique can not determine the parental origin of trisomy among Down patients. Molecular approach using microsatellite markers is the technique of choice to determine parental origin of Down syndrome. Two molecular markers of human chromosome 21 have been used in this study for initial determination of maternally originated trisomies and to evaluate recently developed hypothesis relate to methylenetetrahydrofolate reductase gene (MTHFR) mutation (677 C to T) as a candidate risk factor in Down syndrome. In this way two STRs on Down region of chromosome 21 have been genotyped in 30 families with Down patient referred to clinic and the markers segregation have been determined in each family. In mother originated cases, MTHFR mutation has been screened using PCR-RFLP. This study is unique since the origin of trisomy has been determined initially in each family and the influence of MTHFR mutation has been selectively tested in mother originated families and mothers of down child with paternal origins were not used in the experiment. This helps a better understanding of Down syndrome etiology in Iranian population.

Phenotype/Genotype Correlation of a Cohort of Patients with Papillon-Lefevre Syndrome. *B. Meyer, M. Kambouris, S. El-Samadi, C. Boumah, S. Abu-Amero, A. Butt, A. Al-Fadley, C. Crossner, K. Pedersen, C. Ullbro.* B&MR, King Faisal Special Hosp, Riyadh, Saudi Arabia.

Papillon-Lefevre syndrome (PLS) is an autosomal recessive disease generally characterized by severe periodontitis (PD) and palmoplantar keratoderma (PPK) but heterogeneous in expression. PPK may vary from mild scaly skin to overt hyperkeratosis. Patients with only PD or PPK have been described and in rare instances PD is only mild and of late onset. Loss of function mutations in the cathepsin C gene have recently been identified as the cause of PLS. Approximately 13 nuclear families with 12 different mutations have been reported. The absence of a founder effect and the apparently familial mutations identified have limited the association of specific haplotypes or mutations with disease heterogeneity. We have studied a Saudi Arabian cohort of 20 PLS patients from 12 nuclear families. Haplotype analysis based on homozygosity of affected individuals was performed for the region 11q14-q21 using the markers D11S4147, D11S1795, AFM207ya5, D11S2015, D11S1354, D11S4197, D11S4082, D11S1780 and D11S931. Haplotype analysis identified clear founder effects in this group of patients. All individuals were homozygous at D11S1780 (location of Cathepsin C) with only three alleles (173,183 and 189) being identified. Fourteen patients (8 families) were 183/183, 5 patients (3 families) were 173/173 and 1 individual had a 189/189 genotype. Sequencing exons 1-7 of cathepsin C in the PLS patients identified a 815G-C mutation in exon 6 resulting in an arginine to proline change at amino acid 272 (R272P), to be associated with the 183 allele. Mutations associated with the 173 and 189 alleles are yet to be identified. Initial observations indicate that patients with the 183/183 genotype (R272P) have more severe oral features than those with 173/173. No significant difference in the degree of keratoderma was apparent in these two groups, however ichthyosis was observed in several individuals with the 183/183 genotype. Further phenotype/genotype characterization awaits analysis of additional individuals from the large group of PLS patients present in Saudi Arabia.

Prenatal testing for DFNB1 deafness. *T. ANTONIADI*¹, *A. PAMPANOS*², *M.B. PETERSEN*^{1,2}. 1) Dept of Genetics & Molecular Biology, MITERA Maternity & Surgical Center, Athens, Greece; 2) Dept of Genetics, Institute of Child Health, Athens, Greece.

A single mutation, 35delG, in the gene encoding the gap-junction protein connexin 26 (GJB2) on chromosome 13q11 (DFNB1 locus), has been found in a great proportion of recessive families and sporadic cases of congenital deafness in Caucasian populations. We have previously reported a carrier frequency of 35delG of 3.5% in the Greek population, and the 35delG mutation has been detected in one third of the alleles in Greek patients with sensorineural, prelingual non-syndromic deafness. We here present our recent experience with 29 couples requiring counseling, carrier testing and prenatal diagnosis of DFNB1 deafness. Ten of the couples requested molecular testing after information of the high population carrier frequency, by testing one or both partners, or in two cases direct testing of the amniotic fluid. The 35delG mutation was not detected in these samples. The remaining 19 couples had a family history of deafness. In 4 of these couples, where both partners were carriers of 35delG, they proceeded to prenatal testing (chorionic villi or amniotic fluid). Three fetuses were found heterozygotes for the mutation and one was negative. Deaf and hearing people often have different views and beliefs about genetics. In contrast to the medical model which considers deafness to be a pathological condition, many deaf people define themselves as being part of a distinct cultural group, with its own language and customs. There are so far no surveys about attitudes towards carrier testing and prenatal diagnosis of genetic deafness among the hearing population. Our limited experience from hearing parents having a deaf child shows that they request prenatal diagnosis to avoid a second deaf child. The extent to which people consider a condition to be serious depends on the culture, socioeconomic status, religion and personal experience.

Hypermobility Type (HT) Ehlers Danlos Syndrome (EDS) and Pregnancy: A Case Report. *S.M. Carter¹, R. Einy¹, P. Bobby¹, R. Marion².* 1) OB/GYN, MMC, Albert Einstein Col Medicine, Bronx, NY; 2) Pediatrics, MMC, Albert Einstein Col Medicine, Bronx, NY.

EDS, a heterogeneous group of connective tissue disorders, is associated with a number of complications during pregnancy, including easy bruisability, excessive bleeding, orthopedic problems, stroke and sudden death. In addition, affected women are at risk for premature rupture of membranes (PROM) leading to fetal loss or prematurity. Since the severity of complications is related to the type of EDS present, it is imperative that women receive accurate diagnosis, and, when diagnosis is made prior to pregnancy, that they receive preconceptional genetic counseling and pregnancy management geared to the complications common to that form of the disorder. We report on the management of a woman who meets the criteria of the HT form of EDS. The proband, a 29- year-old primigravida, was initially seen during the first trimester because of a long history of easy bruisability, chronic joint pain and recurrent dislocation of the shoulders. On exam, she was noted to have hyperextensible skin that was velvety in texture, markedly hypermobile joints, and on auscultation of the precordium, a Grade I/VI systolic murmur. A maternal echocardiogram revealed trace mitral and tricuspid regurgitation. Following diagnosis, the proband has been followed in high risk obstetrics clinic. Serum marker screening and a sonogram for fetal anomalies were normal. Because of concerns regarding PROM, bed rest was recommended following the 25th week of gestation. The pregnancy is currently ongoing at 36 weeks. Management and outcome will be detailed. We are aware of only 2 previously reported cases of pregnancies in women with HTEDS. These cases, as well as the present one, will be compared.

Prenatally Diagnosed Bowed Long Bones Associated with Non-lethal Osteogenesis Imperfecta. *D. Myles Reid¹, A. Toi¹, M. Silver², R. Lachman³, M. Thomas¹, A. Pai¹, D. Chitayat¹.* 1) Prenatal Diagnosis and Medical Genetics, Mount Sinai Hospital, Toronto, Canada; 2) The Hospital For Sick Children, Toronto, Canada; 3) Cedars-Sinai Research Institute, Los Angeles, California.

Prenatal detection of non-familial bowed long bones on fetal ultrasound presents a difficult diagnostic and counselling dilemma. The differential diagnosis ranges from severe conditions like campomelic dysplasia to benign isolated bowed legs. We report two fetuses that presented prenatally with bowed long bones and were found post-termination to have non-lethal non-familial osteogenesis imperfecta (OI). Case 1: Our patient was a healthy 24-year-old with no history of consanguinity or OI. An ultrasound at 25 weeks gestation demonstrated bowed femora, more pronounced on the left, and bowing of the tibiae and fibulae which measured below the 5th centile in length. No other fetal abnormalities were detected. The differential diagnosis of femoral and tibial bowing was discussed and the couple chose to terminate the pregnancy. The fetal karyotype was 46,XX. Bone histopathology showed a normal growth plate with hypercellular bone and poor organization of collagen bundles. Fetal X-rays showed severe osteopenia. DNA analysis of COL1A1 and COL1A2 revealed a *denovo* heterozygous G->A substitution in exon 19 of COL1A2 converting Glycine-238 (GGT) to Serine (AGT). Case 2: The patient was a healthy 33-year-old with no family history of OI. Ultrasonography at 18 weeks gestation revealed a short and bowed left femur. A femoral fracture was suspected. Visibility of the brain was suggestive of hypomineralization of the skull. The karyotype was normal, 46,XY. The couple chose to terminate the pregnancy. Postnatal skeletal X-rays showed thin skull bones, generalized hypomineralization and angular bowing of the left femur. Histopathology revealed a healing fracture. DNA analysis detected a heterozygous mutation in exon 13 of COL1A1 causing premature termination. Non-lethal OI should be included in the differential diagnosis of fetal bowed long bones. Confirmatory DNA analysis should be considered as it may provide important information for future pregnancies.

Defect in Type I Collagen in a Mother and Child with Apparent Kyphomelic Dysplasia. *N.H. Robin¹, M. Johnson¹, S. Heeger¹, S. Morrison², P. Byers³.* 1) Dept Genetics, Case Western Reserve Univ, Cleveland, OH; 2) Dept Radiology, Cleveland Clinic Foundation, Cleveland OH; 3) Dept Pathology, Univ Washington, Seattle WA.

Introduction: Kyphomelic dysplasia (KD) characterized by multiple skeletal anomalies including severe femoral bowing in the fetal/neonatal period which resolves with age. Long-term prognosis is usually favorable, with normal development and mild short stature. KD is rare, and its existence as a single entity has been questioned. Autosomal recessive inheritance has been suggested by reports of siblings born to unaffected parents. However, no studies investigating the genetic basis of KD have been reported. Clinical report : GW presented at 26 wk gestation after fetal ultrasound identified bilateral femoral shortening and bowing, with no other skeletal anomalies. GW reported that she was born with severely bowed legs that spontaneously resolved. She had records and radiographs that confirmed her report, which we initially doubted because she had an entirely normal physical exam. In 1967 she was diagnosed with osteogenesis imperfecta (OI) vs. hypophosphatasia. We felt KD was the correct diagnosis based on the resolved femoral bowing. However, because of the suspicion of OI, type 1 collagen synthesis testing was done. Cultured dermal fibroblasts produced populations of collagen types I and III with delayed motility, consistent with a COL1A1 or COL1A2 gene mutation. This pattern is associated with OI types III and IV. Molecular mutation analysis is pending. At birth the baby boy manifest femoral bowing and shortening bilaterally, with normal findings in the remainder of the skeleton. Discussion: Here we report on a mother and son with findings suggestive of KD in whom a type I collagen defect was identified. Neither had findings suggestive of OI. This suggests that some cases of apparent KD may in fact represent an OI variant. In addition, this report has obvious significance for prenatal counseling. If the fetus had been a new mutation, the collagen results would have suggested severe OI, not the relatively mild KD. In this way this report clouds counseling for prenatally detected bent femurs.

Excess miscarriages among maternal relatives in families with neural tube defects. *J. Byrne*^{1,2}. 1) Ctr VI, Children's Natl Medical Ctr, Washington, DC; 2) Boyne Research Institute, Drogheda, Ireland.

Previous studies have suggested that inheritance of neural tube defects (NTDs) does not follow a Mendelian pattern: inheritance is more likely matrilineal. Thus far, a gene for NTDs has not been identified and thus, the mechanism of matrilineal inheritance is unknown. Epidemiologic characteristics of NTD sibships include excess miscarriages, excess children with other birth defects and excess children with NTDs. Excess recurrences of NTDs has also been shown excess among other relatives, especially maternal relatives, thus providing the primary evidence for matrilineal inheritance. Maternal relatives may also show more subfertility and more childlessness (*Amer J Med Genet* 66, 303, 1996; in press, 2000). Evidence of altered reproductive patterns among uncles and aunts of the child with an NTD was sought by interviewing the uncles and aunts directly. This abstract reports preliminary results of the first 115 interviews with uncles and aunts in 16 Irish families who have already participated in our studies. Analyses of these data indicate that miscarriages were more likely among maternal relatives than among paternal relatives. Specifically, 15.0% of 153 pregnancies of maternal relatives ended in miscarriage compared to 7.9% of 140 pregnancies to paternal uncles and aunts ($p=.03$). In addition, maternal uncles were more likely to be childless than maternal aunts ($p=.01$) and maternal first cousins were more likely to have birth defects of all types ($p=.02$), than paternal first cousins. The biological mechanism resulting in matrilineal inheritance in NTD families may involve some form of genomic imprinting. Results from this preliminary analysis suggest that if these epidemiologic characteristics are interpretable as the action of the underlying gene(s), then its (their) effects may be detected within the extended family.

EVALUATION OF THE PRENATAL DIAGNOSIS OF NEURAL TUBE DEFECTS BY FETAL ULTRASONOGRAPHIC EXAMINATION IN DIFFERENT CENTRES ACROSS EUROPE. *P. BOYD², D.G. WELLESLEY², HEK. DE WALLE², R. TENCONI², S. GARCIA-MINAUR², GRJ. ZANDWIJKEN², C. STOLL¹, M. CLEMENTI².* 1) Génétique Médicale, Hôpital de Hautepierre, Strasbourg, France; 2) The EUROSCAN Study Group.

Ultrasound scans in the midtrimester of pregnancy are now a routine part of antenatal care in most European countries. Using data from registries of congenital anomalies a study was undertaken in Europe. The objective of the study was to evaluate prenatal detection of Neural Tube Defects (NTD) by routine ultrasonographic examination of the fetus. All NTDs suspected prenatally and all NTDs, including chromosomes anomalies, confirmed at birth were identified from 20 Registries of Congenital Malformation from 12 European countries ; the registries are following the same methodology. During the study period (1996-98) there were 670.766 births. A NTD was diagnosed at delivery in 542 cases. In 453/542 (84%) the lesion was isolated (166 anencephaly ; 252 spina bifida ; 35 encephalocele). Of the 166 isolated cases with anencephaly, 159 (96%) were correctly identified prenatally ; one was missed to scan, two were wrongly diagnosed, four were not scanned (sensitivity 98%). 87% of the prenatal diagnoses were made before 24 weeks gestation ; 142 (86%) of isolated anencephalic pregnancies were terminated, 11% of these after 24 weeks. Of the 252 cases of isolated spina bifida 171 (68%) were correctly identified prenatally, 112 (65%) before 24 weeks gestation. The diagnosis was missed on scan in 60 cases and 21 were not scanned (sensitivity 75%). The mean reduction in birth prevalence because of termination of pregnancy for spina bifida was 49% (range 6-100%). There was a wide variation between centres in prenatal detection rate (33-100%), termination of pregnancy of prenatally diagnosed cases (17-100%) and gestation both at diagnosis and termination of pregnancy. In conclusion a high prenatal detection rate for anencephaly was reported by all registries although some diagnoses were made late in gestation. There is a large variation in prenatal detection and termination rates for spina bifida between centres, reflecting differences both in policy and culture.

Early prenatal diagnosis of type III Lissencephaly. *S. Patrier*¹, *A. Rossi*², *C. Fallet-Bianco*³, *S. Chouchene*¹. 1) Lab anatomie pathologie, CHG Les Feugrais, Elbeuf, France; 2) EFS-Normandie, Bois-Guillaume, France; 3) Hopital Ste Anne, Paris, France.

We report the case of the first fetus of non-consanguineous and healthy caucasian parents. Ultrasound screening performed at 9 weeks of gestation disclosed a fetus with total akinesia and severe arthrogryposis. Data were confirmed 2 weeks later. Fetal karyotype was normal (46,XY). Pregnancy was terminated at 15 weeks of gestation. Postmortem examination showed : * Extension of lower limbs with clubfoot and flexion of upper limbs, wrist contractures associated with multiple pterygiums (shoulders, elbows, knees). * Diffuse amyotrophy. * Unilateral cleft lip and cleft palate. * No visceral abnormalities were found. Neuropathological examination demonstrated : Smooth brain, normal for age weighing 18 g, hypoplastic cerebellum and brain stem. Histological study mainly showed diffuse neurodegenerative abnormalities of the central nervous system : * Severe neuronal loss of the cortical plate, matrix zones, basal ganglia, brain stem nuclei and spinal cord with aspects of neuronal degeneration (from cell size reduction to karyorrhetic nuclei and cell death). * The cortex was abnormally thin and undifferentiated with anarchic migration in the intermediated zone. * The medulla was hypoplastic with reduction number of motoneurons in anterior horns. * Muscular study demonstrated severe neurogenic hypomyoplasia. * Eyes examination disclosed retinal dysplasia. In conclusion, type III Lissencephaly syndrome, of recessive autosomal transmission, is now a well-known cause of lethal fetal akinesia sequence. The gene is not yet identified. To our knowledge, our observation is the earliest case reported.

Increased rate of a sister-chromatid exchanges in a pregnancy at risk for Bloom syndrome. *E. Passarge¹, S. Plambeck¹, A. Naujoks¹, G.S. Zhu¹, M. Zenker²*. 1) Institut für Humangenetik, Universitätsklinikum, Essen, Germany; 2) Universitäts-Kinderklinik Erlangen-Nürnberg, Germany.

In a pregnancy known to be at risk for Bloom syndrome we analysed cultured amniocytes obtained by transabdominal amniocentesis during the 16th week of pregnancy. Fetal chromosomal analysis revealed a male karyotype 46,XY. The rate of sister-chromatid exchanges was 60.8 per metaphase with a range of 40-75 compared to 5 - 10 normal amniocytes done as a control at the same time. Although the parents had declared after prior non-directive genetic counseling that they wished to terminate the pregnancy, if the fetus would be affected, they reversed this decision subsequently and elected to continue the pregnancy. A male infant was born at term with a birthweight of 2310 g, length 48 cm, and head circumference 32,9 cm. Postnatal chromosomal analysis showed a mean of 38 exchange figures per metaphase from lymphocyte cultures (range 27-51). The infant was examined personally at home by two of the authors (E.P. and M.Z.). No clinical signs of Bloom syndrome were present. The typical phenotype of Bloom syndrome is not apparent. At the age of 7 months, the weight is 5650 g (below the third percentile for Turkish boys), height 64.6 cm (below the third percentile), head circumference 39.2 cm (below the 3rd percentile). The proposita is a 9 year old girl with mild clinical signs of Bloom syndrome except marked pre- and postnatal growth retardation. Her diagnosis has been confirmed by SCE analysis (R.A. Pfeiffer, unpublished data and personal communication). The parents are a consanguineous Turkish couple. A homozygous mutation (T1628A) in the BLM gene has been found (N.A. Ellis & J. German, personal communication). Howell & Davies (Prenat. Diagn. 14:1071-1073, 1994) reported a fetus with increased rate of SCE in a pregnancy at risk for Bloom syndrome which terminated in a spontaneous abortion. Additional aspects of the clinical features, laboratory tests, and genetic counseling will be discussed.

EVALUATION OF PRENATAL DIAGNOSIS OF CONGENITAL HEART DISEASE BY ULTRASOUND : EXPERIENCE FROM 20 EUROPEAN REGISTRIES OF CONGENITAL ANOMALIES. *E. GARNE¹, M. CLEMENTI², C. STOLL¹*. 1) Gntique Mdicale, Hpital de Hautepierre, STRASBOURG, FRANCE; 2) EUROSCAN working group.

Ultrasound scans in the midtrimester of pregnancy are now a routine part of antenatal care in most European countries. using data from registries of congenital anomalies a study was undertaken in Europe. The objective of the study was to evaluate prenatal detection of Congenital Heart Defect (CHD) by routine ultrasonographic examination of the fetus. There were 2456 cases with CHD with an overall prenatal detection rate of 25%. Termination of pregnancy was performed in 293 cases (12%). There was significant variation in prenatal detection rate between regions with the lowest detection rate in countries without ultrasound screening (11%) and the higher detection rate in countries with 3 fetal scans, 40 to 48% ($p < 0,01$). There were 1696 cases with isolated CHD of which 16% were diagnosed prenatally and 5% of pregnancy were terminated (TOP) ; 45% of the 761 CHD with at least one other major extra cardiac malformation (associated cases) were detected prenatally and 21,5% were TOP ($p < 0,01$). Prenatal detection rate and TOP were 40,3% and 22,9% for chromosomal anomalies 51,9% and 30,8% for non chromosomal recognized syndromes and 48,6% and 16,3% for multiply malformed with non chromosomal and non other recognized syndromes, respectively. Only 69 cases (2,8% of the total) were fetal deaths. Prenatal detection rate of CHD varied significantly between countries in relation with fetal ultrasound screening policies and also between countries even with the same screening recommendations. Risk of fetal death is low, which is important to know when counselling parents after prenatal diagnosis of CHD in order to provide ideal care for the patient.

Molecular analysis of Fragile X syndrome in Iranian population. *H. Najmabadi¹, F. Taghizadeh², SH. Teimourian², R. Karimi-Nejad², Y. Shafeghati¹, GH. Baba Mohammady², S.N. Al-Madany², S.S. Hoseiny², MH. Karimi-Nejad².* 1) Genetics research center, University of Welfare and Rehabilitation, Tehran, Iran; 2) Karimi-Nejad Genetics center, Tehran, Iran.

Fragile X syndrome is a common cause of inherited mental retardation demonstrating variable clinical pictures. Diagnosis of fragile X syndrome influences treatment and intervention strategies that can contribute to improvement in outcome. Carrier testing for at risk individuals and prenatal testing allow accurate diagnosis to be provided and empowers families to make informed reproductive decisions. Therefore, due to the high prevalence of Fragile X syndrome we decided to establish a DNA testing centre to analyse the carrier and affected individuals as well as prenatal diagnosis for first time in Iran. Testing for fragile X mutation is based primarily on measuring the length of the Fragile X mental retardation 1 gene (FMR1) region containing the CGG repeat stretch and analysis of the methylation status of the gene (whether the gene is turned off or on). Two separate approaches for DNA testing, PCR, and southern blot analysis were employed. No radioactive material was used in DNA testing procedure. PCR analysis were used to accurately determine CGG repeat range in normal, grey zone and premutation alleles. While the southern blot analysis was performed for identification of full mutations, large premutations and the gene's methylation status. PCR products after polyacrylamide gel electrophoresis were observed by silver staining method. The probe STB 12.3 (Kindly provided by Louis Mandel) was labelled by Digoxigenin and hybridized to the ECOR1 digested genomic DNA that had been transferred to nylon membrane by southern blotting. Seventy six members of twenty five families were studied using traditional cytogenetic approaches and molecular analysis in parallel. The result of these studies demonstrated thirty five males and five females retarded with full mutation. Six females with premutation and twenty individuals with normal CGG repeats. Prenatal diagnosis were performed for eight families. In these studies three affected males, one affected female and four normal fetuses were detected.

Double trisomy [48,XX,+16+22] in spontaneous abortion. *V. Mizhiritskaya*¹, *H. Rimawi*², *T. Chaudhry*², *R.S. Verma*¹. 1) Div. of Mol. Medicine & Gene., Wyckoff Heights Medical Center, Brooklyn-New York Hospital/Weill Medical College of Cornell University, New York and Lutheran Medical Center, Brooklyn, N.Y. and; 2) Lutheran Medical Center, Brooklyn, N.Y.

Autosomal double trisomies are relatively rare but account for as many as 2.5% of first trimester pregnancy losses. The most common trisomy in spontaneous abortuses is trisomy 16, accounting for 31.0% of trisomic conspectuses and 7.3 % of all spontaneous abortions. Trisomy 22 is found in 11.4% of trisomies and 2.26% of spontaneous abortions. Double trisomies show strong association with advanced maternal age. Fetal tissue from a 43 year old women was sent to our laboratory for cytogenetic evaluation. She had a total of 9 pregnancies, six resulted in miscarriages. Double trisomies of chromosome 18 with sex chromosomes are most frequent with an exception to chromosomes 2, 4, 5, 6, 8, 11 13, 14, 15, 16, 17, 18, 20, 21 and 22. To the best of our knowledge, a double trisomy for chromosome 16 in combination with chromosome 22 has been noted in only one earlier case [Reddy, Hum. Genet. 101:339-345, 1997]. This is a highly unusual finding, as these two trisomies are most frequent in single trisomic abortuses. Furthermore, the cause of high incidence of miscarriages in this woman needs to be cytogenetically evaluated because this could be a major cause of increased susceptibility for miscarriages. In general, the recurrence risk for a couple with a previous trisomic conspectus is often cited as 1.0%. But, what is the risk for couples with double trisomies? Should, prenatal diagnosis be offered to every couple who has delivered a previous trisomic abortus?

Detection of chromosomes 21, 18, 13 and XY aneuploidy using multiplex FQ-PCR on human embryos. D.

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Aneuploidy is a major cause of congenital malformations in human. The diagnosis is usually based on cytogenetic analysis on fresh amniotic, chorionic or blood cells. Fluorescent Quantitative-PCR using tri or tetranucleotide repeat is a fast and cost efficient alternative procedure. Here, we report on the results obtained on the screening of our human embryo collection for aneuploidy using a multiplex FQ-PCR. A multiplex PCR for chromosomes 21, 18, 13, X and Y markers, (namely D21S1414, D21S1411, MBP, D13S631, D13S258, HPRT, AMXY) was set up. One fluorochrome (6-FAM, TET or HEX) was assigned to one primer of each marker. DNA was extracted from frozen chorionic tissues of our human embryo collection. Three more markers for chromosome 21 and 18 respectively were tested by simplex PCR when standart markers were non informative. So far, 122 samples have been studied. Trisomy 21, 18 and 13 as well as chromosome X aneuploidy have been excluded in 83 embryos (68 %). In particular, we excluded a trisomy 21 in 98 cases (80%), trisomy 18 in 104 cases (90%) and trisomy 13 in 111 cases (90%). A total of 120 embryos were sexed and monosomy X was excuded in 46/53 females (85%). In conclusion, the 7 multiplex FQ-PCR markers technique described here should help screening for common aneuploidies for research purposes. In addition, this approach could also be used for prenatal diagnosis of genetic disorders and to exclude maternal cell contamination in CVS or amniotic samples.

Confirmation studies of 80 cases in which Level II or Level III mosaicism was detected by prenatal diagnosis.

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We report data from 80 cases in which confirmation studies were obtained at birth; 58 cases were level II pseudomosaicism and 22 cases were level III true mosaicism. Of the 58 cases of level II pseudomosaicism, 20 cases had numerical aberrations (i.e. trisomies 1, 9, 11(2), 12, 13(3), 17, 18(2), 19, 21(4), 22(3), +mar). Another 24 cases involved structural abnormalities: 18 translocations; 1 inversion; 5 unbalanced translocations or deletions. There were 12 cases of sex chromosome aberrations and 2 cases of XX/XY mosaicism. Chromosome analyses from cord blood in 54 cases were normal with 60 to 100 cells being counted. Of the remaining 4 cases, 2 cases of X/XX mosaicism were confirmed in 3% and 8% of the cells while 2 cases of XX/XXX mosaicism were confirmed in 1% and 2% of the cells. There were 22 cases in which mosaicism was detected in 2 or more primary cultures. Autosomal trisomy mosaicism was detected in 9 cases, 3 of which were not confirmed (i.e. trisomies 13, 16 and 20). Sex chromosome mosaicism was detected in 9 cases, 5 of which were not confirmed. Neither the case with mosaicism for a reciprocal translocation nor the case mosaic for an i(20q) was confirmed. Maternal cell contamination was the likely explanation in the 2 cases of XX/XY mosaicism. Reporting Level II pseudomosaicism found in amniotic fluid cultures often presents an ethical and diagnostic dilemma. While this low level mosaicism can be occasionally detected in the fetus, it causes undue anxiety for patients when mosaicism is not confirmed in the fetus. Detailed cytogenetic analysis as well as follow up studies of these cases will be presented.

PRENATAL DETECTION OF CHROMOSOMAL ABNORMALITIES BY FETAL ULTRASONOGRAPHIC EXAMINATION ACROSS EUROPE. *C. DE VIGAN¹, M. CLEMENTI², E. CARIATI², N. BAENA², C. STOLL¹.* 1) Gntique Mdicale, Hpital de Hautepierre, STRASBOURG, FRANCE; 2) EUROSCAN working group.

The objective of the EUROSCAN study group was to evaluate the accuracy of the antenatal detection of chromosomal anomalies by routine fetal ultrasonographic examination in unselected populations. All congenital malformations suspected prenatally and all congenital malformations confirmed at birth were identified from Congenital Malformations Registries including 20 registries from 12 European Countries. These registries are following the same methodology. During the study period 1996-98, 664.269 births were covered including liveborn, stillborn and terminations of pregnancy. The chromosomal abnormalities were classified into 12 groups ; 1783 cases of chromosomal abnormalities were collected ; the main group were Down syndrome (n=1050), trisomy 18 (n=191), Turner syndrome (n=125), trisomy 13 (n=86) and triploidy (n=56). The detection rate by prenatal ultrasonography among the 1683 cases having had a least one scan was 38,9%, with variation by regions and according to the chromosomal categories. The lowest rate was observed in Klinefelter syndrome (5,7%), the highest one in polyploidy (78,6%), it was 26,4% for Down syndrome ; 75,9% of the cases detected by ultrasonography were terminated. In conclusion this study shows that close to 40% of fetal chromosomal anomalies can be detected by routine prenatal ultrasonographic examination. However this detection rate varies according to the regional policy of prenatal diagnosis.

Clinical significance of low level mosaicism for X-chromosomes in couples with fetal loss. *S. Kleyman¹, V. Mizhiritskaya¹, R.S Verma^{1,2}*. 1) Div. of Molecular Med. & Gene., Wyckoff Heights Medical Center, Brooklyn, N.Y.,; 2) New York Hospital/Weill Medical College of Cornell University, New York, N.Y.

Clinical significance of low numbers of mosaicism in routine cytogenetic evaluation remains a dilemma. Most laboratories count only 20 cells in routine cytogenetic evaluations. If an individual case has more than two cells with same chromosome missing or added, a further attempt is made to analyze 50 cells. Are 20 cells sufficient to reach a conclusion for patients who have had more than two fetal losses. We decided to count at least 50 cells in such referred cases; the data are summarized as follows:

Patients	Age	XX	X	XXX	Patients	Age	XX	X
SY	37	44	3	3	CLY	36	49	1
SA	30	45	5		HB	39	97	3
LP	32	48	2		MR	42	48	2
DS	40	46	4		CS	43	74	1
IE	31	48	2		MD	38	48	2

A total of 575 cells were counted from 10 individuals. There were 25 metaphases missing one X-chromosome, while in one individual, there were three metaphases that had 47,XXX counts. About 4.3% cells were 45,X. These findings can no longer be considered fortuitous. They are of great significance for a couple who has had more than two consecutive fetal losses. In such cases, at least 50 metaphases should be counted. This information should be discussed during the counseling session with the patients and amniocentesis should be recommended regardless of the mothers age.

Efficacy of preimplantation genetic diagnosis (PGD) using fluorescent in-situ hybridization (FISH) in balanced reciprocal or Robertsonian translocation carriers undergoing human IVF-ET program. *C.K. Lim¹, J.H. Jun¹, G.J. Song¹, J.W. Kim¹, S.Y. Park², K.H. Kim³, J.Y. Jun³, I.S. Kang³.* 1) Lab. of Reproductive Biology and Infertility; 2) Lab. of Medical Genetics; 3) Dept. of Ob/Gyn, Samsung Cheil Hospital & Women's Healthcare Center, Sungkyunkwan University School of Medicine, Seoul, Korea.

Objectives: This study was performed to evaluate efficiency of PGD using FISH in balanced reciprocal or Robertsonian translocation carriers in human IVF-ET program. **Design:** A retrospective study of balanced reciprocal or Robertsonian translocation carriers undergoing IVF-ET from 1999 to 2000 at our hospital was performed. **Materials and Methods:** FISH was carried out in 30 cycles of 20 couples with reciprocal translocation (19 cycles of 15 couples) and Robertsonian translocation (11 cycles of five couples). Two-color FISH analysis was performed on 259 blastomeres in 25 cycles and three-color FISH analysis was performed on 57 blastomeres in five cycles. Meiotic segregation pattern was examined in three-color FISH. After FISH analysis, the embryos with normal FISH signals were transferred into the uterus. **Results:** In two-color FISH analysis, 55 normal embryos were transferred in 24 cycles and FISH efficiency per embryo was 94.7%. In three-color FISH analysis, normal nine embryos were transferred in four cycles and FISH efficiency per embryo was 96.2%. The meiotic pattern of alternate, adjacent-1, adjacent-2, 3:1 and chaotic segregation were 20.7% (12/58), 17.2% (10/58), 20.0% (11/58), 13.8% (8/58) and 29.3% (17/58), respectively. At present, five pregnancies were achieved after 28 cycles of embryo transfer in 19 couples. Four healthy newborns were delivered at term. Three of them were healthy translocation carriers and one was confirmed as normal. The other is ongoing pregnancy at 36 weeks with a Robertsonian translocation carrier fetus confirmed by amniocentesis. **Conclusions:** The efficiency of PGD using FISH was above 95% and all conceptions after PGD using FISH were confirmed correct as normal or translocation carrier. Two or three-color FISH can be successfully applied for the patients with translocations of chromosomes.

Prenatal diagnosis of beta thalassemia in Iranian population. *R.R. Karimi-Nejad.* Karimi-Nejad Genetics Ctr, Tehran, Iran.

Beta thalassemia is the most common genetic disorder worldwide. About 3% of the world population are carrier of a beta thalassemia mutation. More than 190 different beta thalassemia mutations have been identified. In Iran, where there are at least 2 million carriers, beta thalassemia is a major health problem. Prenatal diagnosis is at present a prime goal for prevention. We have been working on determining the spectrum of beta thal mutations in Iran, for the past 10 years and have come up with a 20 mutation panel that will detect up to 85% of the cases overall, ranging from 70 - 90 % for different geographic and ethnic origins. Use of this panel along with RFLP provides reliable prenatal diagnosis for 90% of the pregnancies again ranging from 80 - 95% according to origin. 292 prenatal diagnoses have been performed during this period for 260 families, resulting in 86 affected major, 130 minor and 56 normal fetuses. In 20 cases we have been unable to reach a definitive diagnosis. In conclusion, for more successful universal prenatal diagnosis in the Iranian population, more work needs to be done in mutation identification, especially in regions where the present panels are less successful.

Postnatal Detection of Beckwith Wiedemann Syndrome: Implications for Prenatal Diagnosis. *O. Reish^{1,2}, I. Lerer³, A. Amiel^{2,4}, E. Heyman^{2,5}, T. Dolphin², Y. Dagan³, D. Abeliovich³.* 1) Genetic Institute, Assaf Harofeh Medical Center, Zerifin, Israel; 2) Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; 3) Genetic Institute, Hadassah Hospital, the Hebrew University, Jerusalem, Israel; 4) Genetic Institute, Meir Hospital, Kefar Saba, Israel; 5) Neonatal Intensive Care Unit, Assaf Harofeh Hospital, Zerifin, Israel.

Beckwith Wiedemann Syndrome (BWS) is an overgrowth disorder characterized by organ hyperplasia, developmental defects and an increased risk of embryonal tumors. We present here two unrelated patients diagnosed postnatally. Both had primordial overgrowth and specifically acceleration in growth pattern from second trimester to large for gestational age at third trimester. Placentomegaly, polyhydramnion and remarkably increased abdominal circumference along with macroglossia were recorded. Hemihypertrophy was congenital in one but developed later in the other who additionally had adrenal hemorrhagic cysts, ureteric, and cardiac anomalies. Partial trisomy of the BWS critical region due to paternal translocation was detected in the latter while paternal uniparental disomy (UPD) was found in the former case. We recommend that whenever these ultrasonic findings are detected prenatally without diabetes mellitus, further evaluation to confirm diagnosis of BWS should be pursued. As amniocyte karyotyping might not detect small rearrangements, fetal molecular studies of BWS critical region should be performed in addition to conventional cytogenetic analysis to detect potential paternal UPD or allelic duplication. An early diagnosis and recognition of BWS is important for a cautious parental counseling and for improved delivery and postnatal management of the patient.

Correlation between abnormal ultrasound findings and abnormal prenatal FISH. *M.J. Pettenati¹, D. Conrad¹, J. Rosnes¹, P.N. Rao².* 1) Wake Forest Univ. School of Medicine; 2) Univ.of California at Los Angeles.

A study was performed on 687 amniotic fluid samples referred for prenatal FISH/cytogenetic analysis to determine which referral reason had the highest incidence of abnormality detection by FISH. The most common singular indication for FISH was: increased +21 risk by MSAFP (33%), abnormal ultrasound [U/S](26%) and AMA(24%). Approximately 9% of these cases had an abnormality detectable by FISH (AneuVysion). There was 100% correlation with cytogenetics. Of the 64 cases with an abnormal FISH, 85% had an abnormal U/S listed as one of the referral indications. To determine which U/S were most highly associated with an abnormal FISH result, we reviewed all prenatal cases referred for FISH with abnormal U/S findings (n=215). Among those cases with a normal FISH (n=157), the most common abnormalities included: hydronephrosis(28%), choroid plexus cysts(23%), abdominal defects(12%), heart defects(8%), limb defects(6%) and IUGR(6%). Single U/S abnormalities were present in 54% of these cases. The most common U/S abnormalities associated with the abnormal autosomal FISH trisomies [13,18,21 (n=46) and triploidy (n=4)] included: heart defects(21%), nuchal thickening(15%), cystic hygroma(15%), choroid plexus cysts(15%), brain abnormalities(14%), IUGR(12%) and limb defects(12%). Multiple U/S abnormalities were present in 71% of these abnormal aneuploid cases. The most common U/S abnormalities associated with sex chromosome aneuploidies (n=12; 45,X: n=9) were cystic hygromas(n=8) and hydrops(n=4).

FISH has proven to be a very useful test for rapid and accurate prenatal testing. This study demonstrates that the chance of detecting an abnormal FISH, using AneuVysion, increases with the number and type of anomalies detected by ultrasound. Studies, such as this, continue to demonstrate an extremely high correlation of FISH and cytogenetic results with abnormal prenatal outcomes. These results would support the position that pregnancy management strategy/decisions could be made when there is an abnormal FISH result in association with specific and/or a combination of abnormal U/S findings.

Limited expression of Fas and Fas ligand in fetal nucleated erythrocytes isolated from first trimester maternal blood. *S. Satoshi*^{1,2}, *O. Samura*¹, *K.L. Johnson*¹, *R.S. Elmes*¹, *Y. Fujiki*², *V.M. Falco*¹, *D.W. Bianchi*¹. 1) Division of Genetics, New England Medical Center, Tufts University School of Medicine, Boston, MA; 2) Department of Obstetrics and Gynecology, University of Tsukuba, Tsukuba, Japan.

Intact fetal cells isolated from maternal blood can be used for gender determination and genetic diagnosis. Recent studies demonstrating a large amount of free fetal DNA in maternal plasma suggest that the circulating fetal DNA may be associated with apoptosis of the fetal cells. In the present study, the potential involvement of Fas and Fas ligand (FasL) with respect to apoptosis induction in the fetal cells from maternal blood was evaluated. We studied the co-expression of Fas and FasL in nucleated red blood cells (NRBCs) that contained the gamma chain of fetal hemoglobin. We flow sorted candidate fetal cells that were gamma positive and Fas or FasL positive or negative, and subsequently analysed them by fluorescence in situ hybridization (FISH) analysis using X and Y chromosome-specific probes. Among all gamma hemoglobin-positive cells, there was a significant difference in the percent of cells expressing Fas versus FasL (4.4 and 12.3, respectively). We found no significant correlation between the total number of fetal NRBCs and gestational age or the presence of Fas-positive and FasL-positive cells. From approximately 7 ml of maternal peripheral blood, most of the confirmed fetal (XY) cells were found in the Fas-negative and FasL-negative sorted population; the average numbers were 12.8 and 15.7, respectively. We conclude that fetal NRBCs express FasL more than Fas, although most fetal NRBCs in maternal blood do not express Fas or FasL. This suggests the absence of a functional Fas/FasL apoptotic system in fetal NRBCs, and that programmed cell death in these cells, which leads to circulating fetal DNA in maternal plasma, probably occurs by another pathway.

Prenatal diagnosis of del(6p23) mosaicism and FRA6A: a case report and review of the literature. *H.A. Yee, F.P. Bernier, J. Chernos.* Medical Genetics, University of Calgary, Calgary, AB Canada.

Prenatal diagnosis of mosaicism for terminal deletions with breakpoints localizing to known rare autosomal fragile sites is an infrequent occurrence. To our knowledge, five such cases have been identified, of which four cases had a normal clinical outcome. Outcome information is not available on the fifth case. We report a unique prenatal case of low level mosaicism for a deletion with corresponding fragile site in regular media but absent in low folate media. Prenatal diagnosis in a healthy 24 year-old primigravida as a result of a positive maternal serum screen showed mosaic 6p23 terminal deletion and a fragile site at 6p23. The pregnancy had been unremarkable. Ultrasounds at 17 and 20 weeks gestation as well as an echocardiogram at 20 weeks did not demonstrate any structural abnormalities. The amniocyte karyotype was 46,XY,del(6)(p23)[6] / 46,XY, fra(6)(p23)[5] / 46,XY[38] with abnormal cells identified in four independent cultures. The fragile site and the breakpoint of the deletion appear to correspond to the recognized rare fragile site FRA6A at 6p23. Studies of fetal cord lymphocytes (by PUBS) demonstrated a low level mosaicism (4%) for the 6p23 deletion but no fragile site in low folate culture. In contrast, both the 6p23 deletion (1.4%) and its corresponding fragile site (7.1%) was present in regular media. FISH analysis with telomeric probes for chromosome 6 demonstrated deletion of the distal short arm in 3.5% of lymphocyte metaphase cells. Parental karyotypes were normal with no fragile site observed using both full regular and low folate media. Parents were counselled that the investigations were consistent with true low level fetal mosaicism for 6p23 deletion and that this deletion has been reported in children with craniofacial dysmorphism, anterior chamber eye defects, hearing loss and heart defects. They have elected to continue the pregnancy. A fragile site can be associated with true low-level mosaicism for a chromosome deletion which may have clinical implications and presents a counseling dilemma when detected at prenatal diagnosis.

Association of thermolabile 5,10-methylenetetrahydrofolate reductase (C677T) with elevated maternal serum alphafetoprotein and normal pregnancy outcome. *N.K. Bjorklund¹, J.A. Evans^{1,2,3}, C.R. Greenberg^{1,2}.* 1) Biochemistry and Med. Genetics, University of Manitoba, Winnipeg, Manitoba, Canada; 2) Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba, Canada; 3) Community Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada.

Introduction: We compared the frequency of the 5,10-methylenetetrahydrofolate reductase C677T thermolabile variant (tMTHFR) in three groups: 1) parents of babies with isolated neural tube defects (NTD), 2) parents of babies whose mothers had elevated midtrimester MSAFP with normal outcome, 3) large unselected newborn population in Manitoba, Canada. **Methods:** Cases and controls were selected on the basis of elevated MSAFP with NTDs and with normal outcomes respectively. They were studied with respect to tMTHFR, dietary and supplemental intake of folate, B12, B6, urban/rural location, ethnicity, baby's gender, mother's age and parity. Collaborators in our department had previously established the baseline allele frequency of tMTHFR in Manitoba (0.2497) by the analysis of 977 anonymous consecutive newborn blood spots collected for routine neonatal screening. **Results:** Twenty five case and 32 control mothers, and 19 case and 18 control fathers participated. When compared to tMTHFR in the Manitoba population, cases did not differ significantly (OR 1.18, $p=0.640$). However, we found that controls did differ significantly from the baseline population frequency (OR 1.42, $p=0.012$). **Conclusion:** Compared to the Manitoba population, our cases had elevated tMTHFR that, while not significant, was consistent with most literature reports. This is, however, the first report that suggests elevated MSAFP in the absence of a NTD outcome is associated with a higher frequency of tMTHFR. Elevated MSAFP is also associated with adverse outcomes in later gestation including prematurity, preeclampsia, placental abnormalities and growth retardation. Our results are suggestive of a possible role for tMTHFR in all these complications of later pregnancy. Prospective analysis of tMTHFR in all mothers with elevated MSAFP and non-NTD outcomes, including third trimester complications, is being pursued.

Evaluation of Prenatal Renal Ultrasonographic Findings in 708,756 Fetuses - An European Study. A.C. *Queisser-Luft*¹, A. *Wiesel*¹, C. *Stoll*², M. *Clementi*³, *EUROSCAN Working Group*³. 1) Children's Hospital, University of Mainz, Mainz, Germany; 2) Service de Gntique Mdicale, CHU, Strasbourg, France; 3) EUROSCAN Working Group.

Objective: Renal abnormalities belong to the most common malformations. This study of the EUROSCAN working group evaluates the prenatal detection rate of renal malformations by routine ultrasonographic examinations in unselected populations of different European countries. Methods: Over a period of three years (1996 - 1998) 708,756 liveborn, stillborn, abortions and terminations of pregnancies were analyzed. Using the same standardized methodology 20 registries of 13 European countries were included. The prenatal detection rate of renal defects in connection with additional major malformations and gestational weeks is demonstrated. Results: In 1,131 fetuses renal malformations were diagnosed (prevalence. 16/10,000). 925 (81.4%) were detected prenatally; in 505 (54,5%) children before the 25th week of gestation. For example 86 (90.5%) of 95 cases with Potter sequence were diagnosed prenatally, 26 (83.8%) of 31 with polycystic kidney disease, 9 (47.3%) of 19 with extrophy of bladder and 19 (70.3%) of 27 with urethral vales. Termination of pregnancy was performed in 62.1% of all cases with potter sequence, in 51.6% with polycystic kidney disease, in 42.1% with extrophy of bladder and in 14.8% with urethral valves. 634 (83.0%) of 763 cases with solitary renal defects and 291 (79.1%) of 368 cases with additional malformations were identified prenatally. 52.7% (194) of associated malformations were diagnosed by the renal defect. The prenatal detection rate varies from 67.7% in countries without to 91% in countries with routine fetal ultrasound examinations. Conclusion: Sensitivity of prenatal ultrasound examinations depends directly on the scan policy of the different European countries. Prenatal identification of renal malformations is an diagnostic factor for additional major malformations and very important for pregnancy management as well as treatment of the neonate.

Dizygotic Monochorionic-Diamniotic Twinning with Discordant Sex and Placental Vascular Anastomoses

Presenting as Twin-Twin Transfusion Syndrome. *R.A. Quintero¹, P.W. Bornick¹, O.T. Mueller², G. Arroyo¹, E. Gilbert-Barnes³, D. Hilbelink⁴, M.H. Allen¹, P.R. Papenhausen⁵, M. Sutcliffe².* 1) Florida Intitute for Fetal Diagnosis and Therapy, Tampa, FL; 2) Clinical Genetics Laboratories, All Children's Hospital, St. Petersburg, FL; 3) Department of Pathology, Tampa General Hospital, Tampa, FL; 4) Department of Anatomy, University of South Florida, Tampa, FL; 5) Labcorp, Raleigh, NC.

A 28 year old gravida 1 was diagnosed with twin-twin transfusion (TTTS) syndrome at 18 weeks' gestation. Ultrasound showed a single anterior placenta, a thin dividing membrane, polyhydramnios in the recipient twin (maximum vertical pocket (MVP) of 8 cm) and severe oligohydramnios in the donor twin (MVP of 1.4 cm). The external genitalia (EG) in the recipient was consistent with that of a female, but the EG in the donor could not be assessed due to severe oligohydramnios. The cervix was 1.3 cm long with funneling requiring an emergency cerclage. During laser treatment for TTTS, 3 arterio-venous communications from donor to recipient were found and photocoagulated. The patient miscarried 3 weeks later. Placental pathology confirmed a monochorionic-diamniotic placenta, with two layers of amnion without intervening chorion at the dividing membrane. However, discordant EG were noted: recipient/female, donor/male. Cytogenetic analysis showed normal 46,XX and 46,XY karyotypes, respectively. Microsatellite analysis using multiple centromeric markers ruled out monozygosity and polar body fertilization and was consistent with dispermic fertilization of two separate ova. We conclude that pregnancy may have resulted from fusion of two fertilized eggs during zona pellucida disintegration. Vascular anastomoses are possible in dizygotic-monochorionic human pregnancies under these rare circumstances.

Prenatal paternity testing: The Role of the Prenatal Genetics Center. *H. Lee¹, A. Shaloub¹, M. Ayoub¹, J. Carmody³, W. Uhlmann⁴, A. Johnson¹, M.I. Evans^{1,2}.* 1) Obstetrics/Gynecology, Hutzel Hospital, Detroit, MI; 2) Center for Molecular Medicine and Genetics, Wayne State, Detroit, MI; 3) Obstetrics/Gynecology, University of Iowa; 4) Medical Genetics, University of Michigan.

Paternity establishment is currently available by DNA analysis using PCR to measure short tandem repeats. Most tests have been done for postnatal litigation, but increasingly women are obtaining paternity information prenatally which can be done by CVS, amniocentesis, or cordocentesis. We report a series of 38 prenatal procedures performed for paternity determination from 1991-2000. Blood or buccal swab samples were obtained from mothers and putative fathers. Twenty women had amniocentesis and 18 had CVS. Each case reported two putative fathers with one man tested. Half of the men tested were primary partners. Result accuracy given by the laboratory was 99.9+%. Nineteen tested men were excluded as the father, 6 of which were primary partners. Sixteen men tested were not excluded, of which 10 were primary partners. Three cases are pending. Patients were asked about reasons for paternity testing in the consultation. Fifteen reported the results would be used to make decisions regarding pregnancy management (including the option of termination): 4 of which were amniocenteses and 11 of which were CVS cases. Thirteen women stated results would be used for information only, that termination of pregnancy was not an option (all of which had amniocenteses performed). The reasons for testing in 10 cases are unknown. In our series, 8 women had testing between 1991-1995, whereas 30 had testing from 1996-2000. The majority of patients seen in our office receive "good news" from the prenatal testing, either excluding the "other man" or not excluding their primary partner (23/36 cases for which outcome is known). Our experience suggests that the majority of patients who are using paternity results to decide on termination of pregnancy choose CVS over amniocentesis. Our data also suggests that the number of women choosing to obtain prenatal testing for paternity establishment is increasing.

Does zona manipulation (ZM) increase the frequency of monochorionic (MC) monozygotic (MZ)

twinning? *M. Vandervorst*¹, *C. Derom*², *I. Liebaers*¹, *R. Vlietinck*², *M. Bonduelle*¹, *R. Derom*³. 1) Brussels Free University, Brussels, Belgium; 2) Katholieke Universiteit Leuven, Leuven, Belgium; 3) Association for the Scientific Study of Multiple Births, Destelbergen, Belgium.

Omitting multicentric studies in which several types of ZM were used and considering only the series of cases coming from one institution and using the same type of ZM, frequencies of 0-1% were found after minimal manipulation like intracytoplasmic sperm injection (ICSI). This report deals with a consecutive series of 54 pregnancies in which the ZM was much more aggressive.

The 54 pregnancies resulted from the development of 68 zygotes: 38 were single, 15 were twin (13 dichorionic [DC] and probably dizygotic [DZ]), 2 MC and 1 was DZ-DC triplet. An opening was made in the zona pellucida (ZP) to remove 1 or 2 blastomeres for preimplantation genetic diagnosis. The structures of the fetal membranes in these 16 multiple pregnancies were:

- in twins, dichorionic-diamnionic: 13, monochorionic-diamnionic (MC-DA): 1, and monochorionic-monoamnionic (MC-MA): 1;
- in the triplet, dichorionic-diamnionic.

The frequency of MZ-MC twinning, 4.4%, is much higher than the 0.5% found in the 1300 iatrogenic multiple maternities of the East Flanders Prospective Twin Survey in which ZM was either not applied or minimal (ICSI). According to current knowledge about the hatching of the blastocyst, twinning at this stage of development starts by partial herniation of the embryo through a hole in the ZM followed by splitting, resulting in DC twinning. MC-DA twinning and certainly the rare form of MC-MA are therefore unexpected.

If confirmed by other studies, the paradoxical increase in late-splitting MZ twinning after ZM questions the classic views on the way the three types of MZ twinning originate.

Genetic Analysis and Counseling of Congenital Defects at the National Institute of Perinathology in Mexico City.

I. Llano, R. Garcia-Cavazos, C. Hernandez, J. Ortiz. Genetics, Inst. Nacional Perinatologia, Mexico D.F., Mexico D.F., Mexico.

The National Institute of Perinathology is one of the most important reference center for the assistance of high risk pregnancies in Mexico City. One of the reasons for admission to the institution is the identification of morphologic alterations of the fetus. The daily examination of the newborn in the nursery area as well as in the fetal medicine meetings allows us the detection of fetuses and newborn undergoing any structural congenital defects which are included within a program in order to determine the etiology of the disorder and give genetic counseling to the relatives. In the institution we reported 11,307 deliveries from 1998 to date and of these the 3.08%, (350 patients) had a congenital defect which were classified as follows: 326(93.14%) were malformations divided in different subclassification like syndromes, associations or sequences. Nine patients (2.57%) with deformation as a part of a sequence or isolated and the same happened with 16 disruptions (4.90%). The birth defects are a health problem in Mexico. Studying and analysing each case on the grounds of their aetiology have let us give the patients a better counseling about the newborn and its outcome as well as to determine the recurrence risk for future pregnancies within the family, it allows us to improve the prenatal diagnosis, give reproductive options and in this way prevent congenital defects. Conclusions: The incidence of congenital defects in a third level institution in Mexico city is similar with the reported incidence in the literature in different countries and with the same distribution in the different categories of the classification of the congenital defects.

X-linked recessive multiple pterygia syndrome: A locus at Xq26. *T.L. Prosen, M.C. Lanasa, W.A. Hogge.* Dept Genetics, Magee Womens Hosp, Pittsburgh, PA.

X-Linked Recessive Multiple Pterygia Syndrome (MPS; MIM:312150) is characterized by fetal hydrops and death, most often in the second trimester. The affected male fetuses have features including pterygia, fetal akinesia, as well as dysmorphic facial features. Although MPS presents a classic constellation of clinical and/or pathological findings, to date, no causative genes or candidate loci have been identified. We have identified a family wherein two sisters have aborted male fetuses in the second trimester. One of these fetuses was examined postmortem and had features characteristic of MPS: multiple pterygia, facial dysmorphism, and severe pulmonary hypoplasia. An X chromosome linkage study was undertaken, where 12 highly polymorphic (CA)_n repeats were typed with an average distance of 13 cM between markers. Nineteen family members in 3 generations, including fetal DNA extracted from 4 spontaneous abortions, were analyzed. Linkage studies in this family identified a candidate region at Xq22.2-Xq27.3. An additional, unrelated individual has been identified who was live-born with multiple pterygia. She carries an apparently balanced de novo translocation 46,X,t(X;14)(q26;q24.3). Because the translocation breakpoint lies within the candidate region from our first family, we conclude that a gene for pterygia exists at this locus. Gene identification studies are currently underway.

A complete first and second trimester multiple-marker screening program using dry maternal blood spots on filter paper. *S.B. Melancon¹, S. Mercier¹, S. Khalife¹, L. Robb¹, L. Chiu¹, P. Miron¹, D. Krantz², T. Hallahan², M. BenKhalifa¹.* 1) Procrea Genetic Services, Mount-Royal, QC, Canada; 2) NTD Laboratories Inc., Huntington Station, N.Y., USA.

Prenatal screening for Down syndrome and open neural tube defects using maternal serum markers is well established in the 2nd trimester of pregnancy. However, sufficient data is available to consider moving maternal screening earlier into the 1st trimester and coupling it with an ultrasound (US) nuchal translucency (NT) measurement. Since no such public health service was available in Canada, in January 1999 we began offering to women seen at Procrea Fertility Centers and at other private obstetric practices in Eastern Canada, a complete prenatal screening program: 1st trimester, from 10 to 14 weeks, relying on free b-hCG and PAPP-A in dry blood spots, together with an US NT measurement available on the premises: 2nd trimester, from 14 to 22 weeks, using AFP and free b-hCG in dry blood as well. US dating was recommended. For quality control, the first 300 samples were tested in parallel in reference laboratories: NTD for the 1st and FBR for the 2nd trimesters. The results were highly reproducible. Clients personal and familial genetic data were obtained and informed consent was required prior to risk assessment. Health professionals who wished to take part in the program were offered appropriate theoretical and practical training in prenatal genetics and US screening in association with the Fetal Medicine Foundation (London). Those who complied with the program requirements were invited to provide maternal samples and NTs when appropriate, from patients of all ages, prior to any other prenatal diagnostic procedure. From the 2,444 samples analyzed between 03/1999 and 04/2000, 73% were from the 1st trimester, 90% of which included NT and US-confirmed gestational age. In contrast, only 42% of the 2nd trimester specimens included US-dated gestational age. A higher detection rate and specificity, as well as patients preference for earlier reassurance, strongly favored 1st trimester over 2nd trimester screening.

Early diagnosis of a recurrence of X-linked hydrocephalus by detection of adductus thumbs in a male fetus at the first trimester of the pregnancy. *G. Viot¹, P. Saugier-Veber², AL. Delezoide³, Y. Hillion³, JP. Bernard⁴, MV. Senat⁴, T. Frébourg², Y. Ville⁴, J. Roume¹.* 1) Génétique Médicale, Hôpital de Poissy St Germain, Poissy; 2) Laboratoire de Génétique Moléculaire, Faculté de Médecine et de Pharmacie, Service de Génétique, CHU de Rouen, 76183 Rouen; 3) Service d'Anatomo-Pathologie, Hôpital de Poissy-St Germain, 78300 Poissy; 4) Département de Médecine Foetale, Hôpital de Poissy-St Germain, 78300 Poissy, France.

L1CAM is a neural recognition molecule involved in mediation of axon bundling and neurite outgrowth during embryonic development. Mutations in the *L1CAM* gene, localized in Xq28, are responsible for overlapping clinical spectra including X-linked hydrocephalus due to stenosis of the aqueduct of Sylvius (HSAS), MASA syndrome and X-linked spastic paraplegia (SPG1). Here, we report the detection of adductus thumbs during the first trimester of the pregnancy as the first clinical sign of HSAS in a male fetus. The mother's history was marked by the termination of her third pregnancy after ultrasonographic detection at 32 weeks of a severe ventricular dilatation, corpus callosum agenesis and adductus thumbs. The foetopathological study confirmed the ultrasound findings and revealed stenosis of the aqueduct of Sylvius and hypoplastic pyramidal tract. Molecular analysis of the *L1CAM* gene in the foetus and in the mother led to the identification in exon 14 of a novel missense mutation (Tyr589His) affecting a key amino-acid for the conformation of the sixth extracellular immunoglobulin domain. During the next pregnancy, ultrasound evaluation allowed the detection of adductus thumbs in a male fetus at 12 weeks. No ventricular dilatation, neither corpus callosum agenesis could be detected at this term. Prenatal diagnosis on chorionic villi led to the detection of the Tyr589His mutation in this fetus. The pregnancy was terminated at 18 weeks. This observation demonstrates that adductus thumbs, which result from extensor muscle aplasia, can constitute the first clinical sign of HSAS during the first trimester of the pregnancy before the development of ventricular dilatation and can be detected as early as 12 postmenstrual weeks using transvaginal ultrasonography.

Physical Mapping of a Novel Sub-Microscopic Inversion-Deletion Causing Skewed X Inactivation and Recurrent Spontaneous Abortion. *M. Lanasa*¹, *W.A. Hogge*², *T.L. Prosen*², *C.J. Kubik*², *E.P. Hoffman*^{1,3}. 1) Dept Biochem & Molecular Gen, Univ Pittsburgh Sch Medicine, Pittsburgh, PA; 2) Ob, Gyn & Reproductive Sci, Magee-Womens Hospital, Pittsburgh PA; 3) Center for Genetic Medicine, Childrens National Medical Center, Washington, DC.

The identification and characterization of X chromosome genes and loci associated with non-random X chromosome inactivation has, of late, been the subject of significant interest. Previously, we described a 50 member pedigree in which 16 females show extremely skewed X inactivation and a statistically significant increase in the frequency of recurrent spontaneous abortion (RSA) (Pegoraro et al, AJHG 61:160-170). Here, we describe the genomic novel rearrangement in this family, via genomic library construction, sequencing and EST mapping. We identified a sub-microscopic inversion-deletion in distal Xq28. The initial event was the common Hemophilia A inversion between homologous repeats in distal Xq28. This was then followed by a deletion in which a large portion of the inverted region (~500 kb) was lost. This rearrangement is inherited as a cell-autonomous recessive lethal defect, causing both skewed X inactivation and RSA. To determine genes lost in this rearrangement, 19 Xq28 ESTs were mapped to a YAC contig assembled across the deleted region. We identified 6 transcripts that map within the deletion. One of these, subunit 3 of Prefoldin (PFDN3, VBP1), is known to be a recessive-lethal in yeast, consistent with a cell-autonomous lethal in female cells. However, SSCP screening of 50 unrelated females with skewed X inactivation did not identify any single gene PFDN3 mutations. The characterization of this family with the unique combined clinical/molecular phenotype of RSA and skewed X inactivation led to a large population based case-control study to determine if X chromosome defects are a significant cause of RSA in the general population. To date, we have found that 15% (15 of 105) of women with RSA show X inactivation skewing >90%, while only 1% (1 of 100) of primiparous controls show this level; suggesting that X chromosome defects are a significant cause of RSA in the general population.

Isolating fetal cells in cultures from maternal blood: no differential response of fetal and adult erythroid progenitors to erythropoietin. *R.M. Bohmer, K.L. Johnson, D.W. Bianchi.* Dept Pediatrics, Tufts Univ Sch Medicine, Boston, MA.

Fetal cells from maternal blood can serve as a source of DNA for prenatal genetic diagnosis. We are trying to amplify and isolate fetal cells in cultures from maternal blood. Fetal erythroid progenitors in culture express only fetal hemoglobin (F+A-) and can be distinguished from most of their adult counterparts which express either only adult hemoglobin (F-A+) or a combination of both (F+A+). However, some adult cells are F+A- like fetal cells. While their proportion of total adult cells is small, their number still overwhelms the extremely rare fetal cells, thereby limiting the purity of flow-sorted fetal cells. Following a previous report indicating a differential dependence of fetal and adult erythroid cells on erythropoietin (EPO), we used the unifying condition of co-culture to test whether the growth and survival of fetal and adult F+ cells followed a different dose response, either in early or late-stage cultures. At extremely low EPO concentrations (< 0.01 U/ml), where both fetal and adult cells died rapidly, fetal cells showed a slightly higher survival rate. However, in the range of relevant EPO concentrations that permitted erythroid cell growth and survival, there was no significant differential response. We further tested for differences in the time course by which early erythroid progenitors become EPO dependent, using the approach of delayed EPO addition. Again, no significant difference between fetal and adult cells was found. We conclude that EPO is an essential cytokine for both fetal and adult erythroid precursors, and that manipulating its concentration or timing can not serve as a tool to achieve a selective growth or survival of fetal erythroid cells in cultures from maternal blood.

PRENATAL DIAGNOSIS OF LIMB REDUCTION DEFICIENCIES BY FETAL ULTRASONOGRAPHIC EXAMINATION IN EUROPE. *A. WIESEL¹, C. STOLL¹, A. QUEISSER-LUFT², U. FROSTER²*. 1) Gntique Mdicale, Hpital de Hautepierre, STRASBOURG, STRASBOURG, FRANCE; 2) EUROSCAN, study group.

Ultrasound scans in the midtrimester of pregnancy are now a routine part of antenatal care in most European countries. Using data from registries of congenital anomalies a study was undertaken in Europe. The objective of the study was to evaluate prenatal detection of limb reduction deficiencies (LRD) by routine ultrasonographic examination of the fetus. All LRDs suspected prenatally and all LRDs, including chromosome anomalies, confirmed at birth were identified from 20 Registries of Congenital Malformations from 12 European countries ; these registries are following the same methodology. During the study period (1996-98) there were 709.030 births, including 7758 cases with congenital malformations. Two hundred fifty cases of LRDs with 63 (25,2%) termination of pregnancies were identified including 138 cases with isolated LRD, 112 cases with associated malformations, including 16 cases with chromosomal anomalies and 38 cases with non chromosomal recognized syndromes. If more than one LRD was present the case was coded as complex LRD. The prenatal detection rate of isolated terminal transverse LRD was 22,7% (22 out of 97), 50% (3 out of 6) for proximal intercalary LRD, 8,3% (1 out of 12) for longitudinal LRD and 0 for split hand/foot ; for multiply malformed children with LRD those percentages were 46,1 (30 out of 65), 66,6 (6 out of 9), 57,1 (8 out of 14) and 0 (0 out of 2), respectively. The prenatal detection rate of LRDs varied in relation with the fetal ultrasound screening policies (no routine scan, 1, 2 or 3 routine scans) from 20,0% to 64,0%.

Mucopolipidosis III type C: Molecular prenatal diagnosis of the disease. *T.C. Falik-Zaccai^{1, 2}, A. Raas-Rothschild³, R. Bargal³, G. Bach³, Z. Borochowitz¹, M. Zeigler³.* 1) Simon Winter Inst Hum Gen, Bnai-Zion Med. Ctr, Haifa,; 2) Sherutai Briut Clalit, Haifa,; 3) Dep of Hum Gen, Hadassah Hebrew Univ. Hospital, Jerusalem, Israel.

Mucopolipidosis III is a rare autosomal recessive lysosomal storage disease resulting from defective mannose 6-phosphate-dependent lysosomal enzyme trafficking. In variant MLIH, complementation group C (MLIHC), N acetylglucosamine-1 phosphotransferase (GNAcPT) is selectively deficient when assayed with lysosomal enzyme acceptors. The g sub-unit of the GNAcPT was recently found to play a major role in lysosomal hydrolase recognition, and its corresponding gene was mapped and the cDNA cloned. A single mutation was identified so far in three unrelated families with MLIHC. We have studied 5 patients, ages 3 to 38 years from a large Arabic Moslem kindred who presented as infants with stiffness of hands. They have progressed slowly to present limited movements of arms, legs and shoulders, short stature; kyphoscoliosis, and dysostosis multiplex of hip joints, coarse faces, normal intelligence and learning difficulties. Biochemical analysis of lysosomal enzymes were compatible with the diagnosis of MLIH. Affected individuals were homozygous for the intragenic marker D16S3024 and for the previously described mutation, ins. C at codon 167 in the g sub-unit of GNAcPT gene. A couple whose daughter was diagnosed with MLIHC and who was extremely concerned about the expected morbidity suffered by the wife's affected sister has requested prenatal diagnosis via CVS. The fetus was found homozygous for the haplotype and the mutational SSCP pattern of MLIHC in this family. Diagnosis was confirmed by markedly reduced lysosomal enzymes activities in cultured villi. The parents have elected to continue the pregnancy. This is the first report of molecular prenatal diagnosis for MLIHC. Understanding the molecular basis of MLIHC enables us to identify carriers and couples at risk, and provide accurate genetic counseling and early prenatal diagnosis of this non-treatable disease. The recurrent mutation in the 4 unrelated families from Israel might represent a mutational hot spot in the g sub-unit of GNAcPT gene.

Differential gene expression in placentas of Down syndrome fetuses. *J.C. Ferreira^{1,3}, P. Dar¹, D. Khabele¹, B. Funke², B.E. Morrow², H. Nitowsky¹, S.J. Gross¹.* 1) Obstet., Gynec., Women's Health Dept; 2) Molecular Genetics Dept., Albert Einstein College of Medicine, Bronx, NY; 3) Obstet., Gynec. Dept., Hospital Garcia de Orta, Almada, Portugal.

Maternal serum multiple marker screening is currently the standard of care in the second trimester to identify pregnancies at risk for fetal aneuploidy. However, this test is burdened with a significant false positive and false negative rate. Expression microarrays now afford the opportunity to identify genes that are differentially expressed in Down syndrome (DS) placentae and indirectly identify proteins that may be used in the development of more specific screening tests. For this study total RNA was extracted from trisomic 21 placentas (test samples) and euploid control placentas (16 to 24 weeks gestation) obtained through our Institutions Fetal Tissue Repository. The RNA obtained from 7 test samples, 7 matched and from 7 non-matched controls were pooled. A microarray including 8,976 ESTs was used. Four experiments were performed: 1) Trisomic pooled samples were hybridized to the array with matched controls 2) Same as 1) but using non-matched controls; 3) Trisomic samples were split and hybridized with each other; 4) The matched and non-matched controls were hybridized with each other. After hybridization, slide scanning and data extraction, genes over or under expressed in experiments 1 and 2 and equally expressed or with opposite expression in experiments 3 and 4 were selected. PCR products obtained from the corresponding clones were used as probes for individual sample testing by Northern blots. We found seven ESTs/genes corresponded to the selection criteria. Probes were obtained from 4 of the 7 clones. Two of the four Northern blot hybridization reactions evidenced meaningful results, in which trisomic cases (5 samples) were consistently over expressed in comparison with the 5 individual controls. These initial results indicate the feasibility of this global approach to differential gene expression in DS. Further experiments will be required to confirm the consistency of these findings in individual samples, followed by the characterization of the relevant proteins.

Effect of ethnicity on first-trimester Down syndrome screening. *T.W. Hallahan¹, D.A. Krantz¹, P.D. Buchanan², J.W. Larsen³, J.N. Macri¹.* 1) NTD Laboratories, Huntington Station, NY; 2) GeneCare Medical Genetics Center, Chapel Hill, NC; 3) The George Washington University, Medical Center, Washington, DC.

Ethnic-specific analyte medians and IDDM adjustment are routinely used in second trimester screening for ONTD and Down syndrome. To determine if adjustments might also be necessary in first trimester screening, we analyzed 4,531 non-diabetic and 26 insulin-dependent singleton pregnancies between 9 and 13 weeks. Of these pregnancies, 3,393 had NT performed. The table below shows median weight-adjusted MOM values and *P* values based on a rank-analysis of variance.

	N	Free Beta	PAPP-A	No. of NT	NT
Caucasian	4137	1.00	1.00	3059	1.00
African Amer.	145	1.02 (.456)	1.20 (<.001)	119	1.07 (.011)
Native Amer.	4	0.73 (.076)	1.06 (.380)	4	1.48 (.065)
Asian Ind.	42	1.03 (.495)	1.10 (.095)	28	1.02 (.376)
Hispanic	132	0.97 (.068)	0.99 (.486)	104	1.09 (<.001)
Oriental	71	1.15 (<.001)	1.38 (<.001)	60	1.14 (<.001)
IDDM	26	0.88 (.240)	0.94 (.193)	19	1.09 (.212)

The data indicate that as with second trimester screening ethnic-specific medians will likely be required in first trimester screening although more data is needed to precisely develop these medians. IDDM adjustment does not appear necessary in first trimester screening.

Use of dried blood samples in ONTD and Down syndrome screening. *D.A. Krantz¹, T.W. Hallahan¹, P.D. Buchanan², J.W. Larsen³, J.N. Macri¹.* 1) NTD Laboratories, Huntington Station, NY; 2) GeneCare Medical Genetics Center, Chapel Hill, NC; 3) The George Washington University Medical Center, Washington, DC.

Use of dried maternal blood samples offers significant advantages over liquid samples including: elimination of broken tubes, reduced biohazard and sample degradation and elimination of centrifugation. In 1996, we published an initial report on dried blood screening using free Beta hCG and AFP in which we achieved 75% (6/8) detection of DS and 100% (7/7) detection of ONTD. We present an updated and expanded comparison of our screen positive rates before and after GA revision. Risk cutoffs were 1/400 for ONTD, 1/380 for DS, and 1/500 for T-18.

Macri et al. 1996 Overall Experience		
Total Screened	7,497	75,629
Init. Pos. Rate: ONTD	4.4%	4.0%
Init. Pos. Rate: DS	3.6%	3.5%
Init. Pos. Rate: T-18	N.R.	0.8%
False Pos. Rate: ONTD*	2.7%	2.3%
False Pos. Rate: DS	2.8%	2.5%
False Pos. Rate: T-18	N.R.	0.7%

N.R. = Not Reported, * Results after second specimen collected.

In conclusion, the data indicate that the screen positive rate in our expanded experience with dried maternal blood screening is similar to the initially reported data.

Are dual elevations of a-fetoprotein and human chorionic gonadotropin associated with an increased risk of adverse pregnancy outcome? *K.A. Martin¹, E. Listgarten², B. Oberle¹, D.L. Gray¹.* 1) Ob/Gyn, Washington University, St Louis, MO; 2) Genetics and IVF, Fairfax, VA.

Maternal serum a-fetoprotein (msAFP) and human chorionic gonadotropin (msHCG) have each been associated with adverse pregnancy outcomes. We hypothesize that women with dual elevation (DE) of msAFP and msHCG are at higher risk than women whose serum screen indicates increased risk for Down syndrome or neural tube defects.

The DE group had msAFP and msHCG ≥ 2.0 MoM. Two control groups were identified, the DS group comprising women at increased risk for Down syndrome (risk $> 1/270$) and the AFP group whose msAFP was ≥ 2.0 MoM but msHCG was < 2.0 MoM. Pregnancy outcomes were ascertained by review of in and out patient records, and from patient/physician reporting. Outcomes were compared using student's t-test and Fisher exact test.

The DE group was comprised of 40 women and had significantly higher msAFP and msHCG (4.03MoM and 3.85MoM) than the DS group (0.82MoM and 1.94MoM) and AFP group (2.71MoM and 1.09MoM) with $p < 0.05$. Compared with the DS group, the DE group delivered earlier (35.3 Vs 38.8 weeks, $p < 0.001$), had a higher incidence of intrauterine growth restriction (29.7% Vs 2.8%, $p = 0.005$), fetal distress (16.7% Vs 0%, $p = 0.04$), and oligohydramnios (21.8% Vs 0%, $p < 0.01$). Compared to the AFP group, the only significant difference was an increased risk of delivery at ≤ 36 weeks (42.5% Vs 13%, $p = 0.03$).

Women with dual elevations of msAFP and msHCG are at increased risk for premature delivery, intrauterine growth restriction, fetal distress and oligohydramnios. Only the risk for preterm delivery is significantly increased over that in women with elevated msAFP alone. Therefore, elevated msHCG appears to contribute minimally to the predictive value for adverse outcome. Women with dual elevations should be offered serial ultrasound evaluation and be counselled regarding the increased risk for the identified adverse outcomes.

First trimester maternal serum biochemical and ultrasound screening for Down's syndrome in Finland. M.

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Objective. With the growing recognition of the frequency and importance of congenital disorders and with current social trends toward smaller families and delays in childbearing, prenatal diagnosis has an important role in the management of most pregnancies in the western countries. We wanted to study the efficacy of combined, biochemical and nuchal translucency screening in Finland. **Methodology.** The screening study, PAPP-A and -hCG were offered free of charge and on a strictly voluntary basis in health centers in East and North-Finland. PAPP-A and free b-hCG s were analyzed by Wallac Research Company, Turku, Finland. The laboratory was provided with the diameter of nuchal translucency (if available), The adjusted estimated risk for Down syndrome was calculated according to Wallac. **Results.** A total of 2 515 of pregnant women participated the screening in 1999. The number of pregnant women older than 35 years of age were 17.5% which confirms to the average Finlands pregnancy statistics. Among them there were 8 pregnancies affected by Down syndrome, and 2 by trisomy 18. Seven fetuses affected by Down's syndrome and the fetuses with trisomy 18 were detected on the basis of serum screening. Among the screenees the nuchal translucency was measured in 1.602 cases, and among them there were 5 pregnancies affected by Down syndrome), and 2 by trisomy 18. Three fetuses affected by Down's syndrome and the fetuses with trisomy 18 were detected on the basis of nuchal translucency screening. Combining the biochemical data and nuchal translucency the test detected four cases of five with trisomy 21. **Conclusion.** Biochemical screening is more efficient if the nuchal translucency is counted in calculating the risk figure. In this way it is possible to increase the sensitivity of detecting Down's syndrome to 85 - 89% and decrease the number of false positive cases to 5% .

Integrated Prenatal Screening in Ontario: A logical next step in screening for Down syndrome. *A.M. Summers, C. Meier, I. Lasis, P.R. Wyatt.* Genetics Program, North York General Hosp, Toronto, Ontario, Canada.

Wald et al. (1999) have described Integrated Prenatal Screening (IPS) for Down syndrome (DS) combining nuchal translucency (NT) and pregnancy-associated plasma protein-A (PAPP-A) in the first trimester with triple or quadruple marker screening in the second. If three markers are used in the second trimester, IPS has been estimated to detect 83% of DS cases with a 1.5% false positive rate (FPR).

In Ontario, maternal serum triple marker screening (MSS) has been offered as part of a coordinated provincial programme since July 1, 1993. A major criticism of MSS has been the high false positive rate. To date, nuchal translucency screening has been offered in only a few centres in Ontario and until recently, first trimester biochemical markers had not been developed. Over the past decade, the uptake of CVS has been declining. Therefore, the predicted FPR made IPS a logical prenatal screening alternative for women in the province.

IPS was offered to each woman who would have previously been offered MSS and who presented to her health care provider before 13w6d gestation with a viable pregnancy. The woman had to agree to wait for her second trimester test before receiving a result. All screen positive women (term risk > 1:200) were offered genetic counselling, amniocentesis and a detailed ultrasound. All pregnancies will be followed up. At present, IPS is limited to women delivering at North York General Hospital.

Since November 1999, 493 women have completed IPS and 22 were screen positive. Of these 22 positives, three have been true positives for Trisomy 18 and one for Down syndrome. This gives a positive rate of 4.55%. The expected positive rate for IPS in this population (mean age = 33 years) would be slightly over 2.5%. If this population of women had chosen triple marker screening, the positive rate would have been 13.1%.

Preliminary results show that IPS is a clear improvement over MSS in terms of false positive rate.

The molecular Analysis of trinucleotide expansion, Myotonic Dystrophy in Iranian Patients. *S.S. Hossini¹, K. Kahrizi¹, S.M.F. Mousavi¹, H. Najmabadi^{1,2}.* 1) Welfare and Rehabilitation University, Tehran, Iran; 2) Karimi-Nejad pathology and Genetics center.

Myotonic Dystrophy (MD) is the most common form of adult muscle dystrophies with an estimated prevalence of about 1 in 8000, which is inherited by autosomal dominant pattern of trinucleotide (CTG) repeats. In 3' untranslated region of the Serine-Threonine protein Kinase gene located on chromosome 19q13.3. Due to similarity in clinical picture with the other dystrophic patients and the lack of diagnostic methods, we decided to establish molecular analysis for DM gene. A combination of PCR and Southern analysis techniques was used. PCR products were visualised by silver staining. DNA probe (kindly provided by M.Alwazzan) was labelled by Digoxigenin in southern analysis. Over twenty families were tested of which most were negative for DM gene mutations.

prenatal diagnosis of duchenne and becker muscular dystrophy in Iranian population. *F. Pourfarzad^{1,2}, R. Karimi Nejad¹, Y. Shafegati^{1,2}, S. Kheradmand Kia¹, A. Nadji¹, S. Jalil Nejad¹, M.H. Karimi Nejad¹, H. Najmabadi^{1,2}.* 1) Karimi Nejad path and Genetic center , Tehran, Iran; 2) Genetic research center, welfare and rehabilitation uni, Tehran, Iran.

Duchenne and Becker Muscular Dystrophies (DMD / BMD) are X-linked disorders affecting 1 out of 3500 and 1 out of 18000 live male birth, respectively. Both DMD/BMD result from heterogeneous mutations in the dystrophin gene and in about 65% of the Cases one or more exons of the gene are deleted . The reaming cases are due to point mutation. One third of cases arise from new mutation and two third are familial. To determine the range of deletion in Iranian patients and prenatal diagnosis, a deletion screening and linkage analysis were preformed in a group of 23 unrelated DMD/BMD patients. Three sets of multiplex PCR were used to screen for 20 most frequent deletion of dystrophin gene. Three intragenic RFLPs (pERT 87 .15 / Bam HI, pERT87.8/Taq I, pERT 87.15/ Xmn I) and two CA repeats (3Dys and NM72/73) which have the most hetrozygosity Frequency, were used to carrier detection and linkage analysis. Deletions were detected in 52% of patients. Most of the deletions were exons 48, 49 & 50 (~14%) and the number of deleted exon varied from one to nine. So far six prenatal diagnosis have been performed.

Single cell analysis of --^{SEA} determinant of α -thalassemia for preimplantation genetic diagnosis and avoidance of Hb Bart's hydrops fetalis syndrome. S.S. Chong^{1,2,3}, A.S-C. Tan¹. 1) Dept Pediatrics, Nat'l Univ Singapore; 2) Dept Ob/Gyn, Nat'l Univ Singapore; 3) Mol Diagn Ctr, Dept Lab Medicine, Nat'l Univ Hospital.

Background: Hb Bart's Hydrops fetalis represents a serious healthcare problem in many parts of Southeast Asia. This condition occurs when parents who are carriers of a double α -globin gene deletion *in cis* both contribute the deletion to an offspring; the resultant lack of α -globin genes in the fetus causes severe anemia, and thus hypoxia, heart failure, and hydrops fetalis. Such pregnancies also involve increased risks of serious maternal complications, including hypertension and preeclampsia, polyhydramnios, and post-partum hemorrhage. Preimplantation genetic diagnosis (PGD) offers a way to avoid affected pregnancies by selective transfer and implantation of only unaffected embryos. The procedure involves biopsy of single blastomeres from 8-10 cell stage embryos obtained by IVF/ICSI, followed by analysis of the isolated blastomeres to detect specific gene defects. Based on the results of the single blastomere analysis, only unaffected embryos are transferred for ensuing implantation and pregnancy. **Objectives:** To develop a single cell molecular assay to detect the most common Southeast Asian α -thalassemia determinant (--^{SEA}), for application to PGD and avoidance of Hb Bart's hydrops fetalis syndrome(--^{SEA}/--^{SEA}). **Methods:** A nested gap-PCR assay to detect normal and mutant alleles was developed and validated on single lymphoblasts of known α -thalassemia genotype (aa/--^{SEA}). Amplimers were designed such that normal and mutant alleles could be clearly and rapidly discriminated on agarose minigels based on amplicon size. **Results:** Nested gap-PCR of heterozygous lymphoblasts (aa/--^{SEA}) consistently yielded the expected normal and mutant amplicons of 832 bp and 582 bp, respectively. Results were obtained within 6 hours. Non-specific amplification products were never detected, and all media and buffer blank tubes were negative. **Conclusions:** We have developed a robust, reliable, and rapid single cell assay to detect the --^{SEA} determinant of α -thalassemia. For at-risk couples, PGD to avoid Hb Bart's hydrops fetalis pregnancies is now possible.

Preimplantation diagnosis for couples with one partner affected by single gene disorder. A. Kuliev, S. Rechitsky, O. Verlinsky, V. Ivakhnenko, C. Strom, Y. Verlinsky. Reproductive Genetics Inst, Chicago, IL.

Preimplantation genetic diagnosis (PGD) is an important option for couples with one of the partners homozygous for single gene mutations, because of 50% risk for producing an affected child. We performed PGD for three such couples, one for phenylketonuria (PKU) with double heterozygous (R408W/Y414C) male affected partner, and two for beta-thalassemia, both with female affected partners, one homozygous for IVS1-110, and the other - double heterozygous, IVS1-6/IVS1-110. In the latter couple, who attempted two subsequent PGD cycles, husband was a carrier of IVSII-745 mutation, while in the former, the same mutation as the carrier female partner (IVS1-110). PGD was based on pre-selection of mutation free oocytes from heterozygous mother for PKU, using sequential first and second polar body analysis, while embryo biopsy was the method of choice in the other two couples. As only half of the embryos from such couples may be expected to have a normal paternal or maternal allele of the beta-globin or phenylalanin hydroxylase gene, only heterozygous embryos were pre-selected for transfer, using multiplex nested PCR analysis. This included simultaneous amplification of 2 linked polymorphic markers together with beta-globin (STR at the 5' end of the gene and HUMTH01) and 3 linked markers for phenylalanin hydroxylase gene (STR in intron 3, RFLP Xmn I in intron 8, and VNTR at the 3'end of the gene). Prior to blastomere testing, performed for both couples at risk for thalassemia, a single sperm typing was done to reveal the haplotypes of the affected and unaffected paternal chromosomes. Pre-selected heterozygous embryos were transferred back to uterus, while those affected were exposed for further PCR analysis confirming polar body or blastomere diagnosis. Of a total of 10 embryos studied for beta-globin gene mutations, 6 contained a normal allele, and transferred back to patients (3 per each couple). In couple with affected PKU male partner, 6 of 11 oocytes tested by both first and second polar body, were mutation free, of which 4 developed into 8-cell embryos and were transferred, resulting in an unaffected pregnancy and birth of 2 healthy children.

Preimplantation genetic diagnosis for p53 tumor suppressor gene mutation. *S. Rechitsky, C. Strom, O. Verlinsky, J. Nefedova, V. Ivakhnenko, A. Kuliev, Y. Verlinsky.* Reproductive Genetics Inst, Chicago, IL.

Preimplantation genetic diagnosis (PGD) has presently been extended for common disorders of later life. These conditions have never been considered as indication for prenatal diagnosis because of potential pregnancy termination, which could not be justified by a genetic predisposition. With current possibility for pre-selection of the embryos free from genetic predisposition, a pregnancy termination is avoided, as only potentially normal pregnancies are established. We introduced PGD for p53 tumor suppressor gene mutations, to pre-select mutation free embryos for transfer, so avoiding an inherited cancer predisposition. PGD was performed for two couples, one with maternally and one with paternally derived p53 tumor suppressor gene mutations (902insC & G524A, respectively). In the latter couple, paternal haplotypes were studied using single sperm analysis. Multiplex PCR was performed, involving p53 gene mutation analysis, together with the linked short tandem repeats (STRs). In two cycles performed for maternally derived 902insC mutation in exon 8, mutant allele was linked to the 3, and normal to the 4 repeats (TAAA) in intron 1. Multiplex PCR analysis for 902insC mutation and linked STRs, performed in the first and second polar bodies, showed that 6 of 10 oocyte with available results were affected based on heterozygous first and normal second polar body. The intron 1 STR analysis in these oocytes revealed allele drop out in the majority of first polar bodies, predicted heterozygous by mutation analysis. Two embryos resulting from mutation-free oocytes were transferred in each cycle, but failed to establish a clinical pregnancy. In two cycles performed for paternally derived mutation G524A in exon 5, the mutant allele was linked to the 3, and normal to the 2 repeats (TAAA) in intron 1. Of 18 embryos with results, amplification of G524A mutation simultaneously with linked STRs revealed 11 affected and 7 mutation free embryos, 2 of which were transferred in each cycle, resulting in an ongoing clinical pregnancy.

Preimplantation genetic diagnosis (PGD) for translocations using nuclear transfer techniques to convert single blastomeres and polar bodies into metaphase chromosomes. *Y. Verlinsky, J. Cieslak, S. Evsikov, V. Ivakhnenko, C. Strom, A. Kuliev.* Reproductive Genetics Inst, Chicago, IL.

PGD for translocations is currently based on interphase FISH analysis of blastomeres, or the first polar body (PB1) testing. However, both of these methods have limitation for complete karyotyping without which pre-selection of normal or balanced embryos cannot be performed with precision. We developed nuclear transfer techniques for visualization of the second polar body (PB2) chromosomes as well as single blastomeres, which were applied for PGD for maternally and paternally derived translocations. A total of 31 PGD cycles were performed, including 13 for paternally and 18 for maternally derived translocations. The latter were done by PB1 and PB2 analysis. To obtain PB2 metaphase chromosomes, PB2 nuclei were injected into enucleated oocytes, activated through fusion and followed by exposure to okadaic acid to induce premature chromosome condensation. To visualize chromosomes from single blastomeres, their nuclei were fused with enucleated or intact mouse zygotes and the resulting heterokaryons fixed at the metaphase of the first cleavage division. Depending on the type of translocation, a combination of whole chromosome painting, telomeric or centromeric probes was used (Vysis), with conclusive results available in 99 (82.8%) of 121 blastomeres, and 121(83.4%) of 145 oocytes tested. 13(11%) of these oocytes with alternate mode of segregation in PB1 had also meiosis II error, resulting in unbalanced embryo that would have been misdiagnosed if the testing was performed on PB1 alone. 8 embryos (5 normal and 3 balanced) resulting from oocytes with chromatid exchange and complex segregation in PB1, would have not been pre-selected for transfer without PB2 analysis. Overall 43 (14.6%) normal and 39 (13.2%) balanced embryos were pre-selected for transfer in 24 (77.4%) of 31 cycles, resulting in 6 pregnancies, of which two resulted in spontaneous abortions, one in healthy delivery and three are ongoing. Conversion of single blastomeres and PB2 into metaphase chromosomes in combination with PB1 FISH analysis allows PGD for any paternally or maternally derived translocation.

Balanced chromosome translocation can be diagnosed in preimplantation genetic embryos. *K. Xu, H. Yuan, Z. Shi, Z. Rosenwaks.* Ctr Reprod Med & Infertility, Cornell Univ Medical Col, New York, NY.

Objectives: Balanced translocations are common (1/625 of general population) and are associated with risks of having miscarriages or malformed and mentally retarded child. Preimplantation genetic diagnosis (PGD) by fluorescent in situ hybridization (FISH) for structural chromosomal analysis has not been extensively studied. The purpose of this study is to determine the feasibility and accuracy of PGD using telomeric probes for detection of balanced translocation in preimplantation embryos. **Materials and Method:** In this study 17 couples (12 female and 5 male balanced translocation carriers) were enrolled. The average age of female partners was 33.3 \pm 4.0. A total of 25 IVF-PGD cycles was initiated from April 1997 to March 2000. A standard ovary stimulation and IVF procedure was used. Day-3 embryo biopsy was performed, followed by FISH using TelVysion probes (Vysis, IL), which allowed the detection of unbalanced specific chromosome segments. Affected chromosomes of these patients included either arms on chromosomes 1, 4, 5, 8, 9, 10, 11, 13, 14, 15, 16, 18, 20, 21. Only chromosomal balanced or normal embryos were transferred on day-4 or day 5. **Results:** A total of 315 oocytes (average: 12.6/cycle) were retrieved and 167 embryos were biopsied. An average of 2.0 chromosomal balanced or normal embryos (ranging from 1 to 4) were transferred in 23 attempts, resulting 11 clinical pregnancies (47.8% per transfer). Three patients delivered healthy babies, 3 miscarried and 5 were ongoing. Karyotyping from two miscarried fetal tissues revealed that one fetus was trisomic 22 (unrelated to the balanced translocation) and the other had a normal karyotype. **Conclusion:** The present study demonstrates that PGD by FISH using telomeric probes for balanced translocation carriers is feasible and accurate. It is an effective alternative for couples who do not want to make a difficult decision to abort an unbalanced fetus. Moreover, PGD may increase the chance to have a child with a normal or balanced karyotype since IVF may provide more embryos for selection from a female reproductive cycle.

Longitudinal fetal DNA quantitation studies in maternal cells and plasma over a 24 hour period. *D.W. Bianchi¹, J.C. Eller¹, L.Y.S. Chang², R.S. Elmes¹, Y.M.D. Lo²*. 1) Div of Genetics, New England Medical Ctr, Tufts Univ School of Medicine, Boston, MA; 2) Dept of Chemical Pathology, Prince of Wales Hospital, Hong Kong.

Background: In normal pregnancies, ~1 fetal cell per 1 mL maternal whole blood is present (*Am J Hum Genet* 1997;61:822-9). Significant amounts of free fetal DNA can also be detected in maternal plasma (*Am J Hum Genet* 1998;62:768-75). We tested the hypothesis that the highest number of intact fetal cells is recoverable in maternal blood at the time of collection. Over time, exposure to the maternal immune system possibly lyses fetal cells, resulting in increased fetal plasma DNA and decreased intact fetal cellular DNA.

Methods: Blood samples were obtained from 5 women (25-33 wks) with known male fetuses. 3/5 were in preterm labor, 1 had complete previa, and 1 was normal. Samples were rushed immediately to the lab (time=0) and a 2 mL aliquot was centrifuged; plasma and cells were frozen separately. Plasma samples were quantitated in Hong Kong using amplification of DYS14 and Taqman PCR. Fetal cellular DNA was measured in Boston using qPCR amplification of Y49a.

Results: Neither plasma nor intact fetal cellular DNA concentration showed a temporal trend. The normal pregnancy had the least fetal plasma DNA and no detectable fetal cellular DNA. Women in preterm labor had more fetal plasma and cellular DNA detected. At each time interval in the same woman, significantly more fetal DNA was found in plasma than in whole blood.

Conclusions: As measured by fetal DNA concentration in maternal plasma, lysis of fetal blood cells does not appear to occur over a 24 hour period. However, due to the significant amount of fetal DNA of presumed placental origin present in plasma of women in preterm labor, additional signals generated by blood cell lysis may be obscured. Our results validate and extend prior data that abnormal pregnancies result in increased fetomaternal transfusion of both cells and DNA.

Prenatal Diagnosis of Dominant Tibial Hypoplasia/Aplasia with Polydactyly and Triphalangeal Thumbs in Consecutive Pregnancies. *S.N. Barringer¹, K.V. Icke¹, E. Frost², M. Curtis³, B.A. Karczeski¹, C.L. Lowery¹, H.B.*

Collins¹, T. Angtuaco². 1) Dept OB/GYN, Univ Arkansas Medical Sci, Little Rock, AR; 2) Dept. of Radiology, Univ Arkansas Medical Sci, Little Rock, AR; 3) Dept. of Pediatric Genetics, Univ Arkansas Medical Sci, Little Rock, AR.

In 1969, McCusick and Eaton reported a family in which members of three generations were affected with bilateral hypoplasia of the tibia as well as polydactyly of the hands and feet. Since then, few other families have been reported with similar defects or total agenesis of the tibia. In these subsequent reports, triphalangeal thumbs and syndactyly were other commonly noted features. At first glance, these cases appear to be similar to the Werner type of mesomelic dysplasia, though anomalies of the gut, central nervous system, and of the heart have been reported in that condition. This may be evidence that a spectrum of skeletal anomalies exist, or of extreme variable expressivity of this autosomal dominant condition. Prenatal diagnosis of this tibial hypoplasia condition was reported in the literature in 1984. We introduce a family in which prenatal diagnosis of the syndrome was ascertained in two consecutive pregnancies. The proband, a 21 year-old woman, was first seen in our prenatal diagnosis clinic in 1999. She presented with bilateral amputation of the lower legs. She underwent several corrective surgeries of both hands for polydactyly and triphalangeal thumbs. Her mother was born with tibial hypoplasia, and did eventually walk with aid of surgery and braces. She, too, had corrected polydactyly of the hands and triphalangeal thumbs. No other systemic anomalies were reported. In the first pregnancy, ultrasound examination revealed bilateral absent tibia, bowed fibulae, and polydactyly of the hands. Additional anomalies noted were left hand syndactyly, triphalangeal thumbs, and possible abnormal left radius. The second pregnancy revealed bilateral clubbed feet, absent left radius, and medial rotation of the left hand. The proband and her family have been counseled extensively regarding recurrence risks.

Simultaneous visualization of fetal cell marker and chromosomal FISH for prenatal diagnosis using fetal erythroblasts in maternal blood: Development of a novel technique. *M. Choolani, H. O'Donnell, C. Campagnoli, S. Kumar, I. Roberts, P. Bennett, N.M. Fisk.* Fetal and Maternal Medicine, Imperial College School of Medicine, Queen Charlottes and Hammersmith Hospitals, London, England.

Fetal erythroblasts enriched from maternal blood should allow first trimester, non-invasive prenatal diagnosis. Heme autofluorescence limits the use of FITC and PE as fluorophores to label the fetal cell marker. Also, current immunoenzymatic methods of fetal cell identification give poor hybridization efficiencies with chromosomal FISH and do not permit simultaneous visualization of the fetal cell identifier and the FISH signal. We describe a novel, rapid and reliable technique that allows us simultaneously to visualize fetal erythroblast morphology, the chromosomal FISH signals and a fluorescent blue dye (7-amino-4-methylcoumarin-3-acetic acid, AMCA) used to tag the anti-epsilon globin antibody which is not expressed in adults. In mixtures of fetal erythroblasts in maternal blood, the technique is sensitive enough to identify one epsilon positive fetal erythroblast amongst 10^5 maternal nucleated cells. The specificity was 100%. The mean difference in relative fluorescence intensity between fetal erythroblasts stained positive for an anti-globin chain antibody and the autofluorescence of unstained cells was greater with AMCA (mean 43.2, 95% CI 34.6-51.9, SD 14.0) as the reporting label compared to FITC (mean 24.2, 95% CI 16.4-31.9, SD 12.4) or PE (mean 9.8, 95% CI 4.8-14.8, SD 8.0). Using our protocol, the median hybridization efficiency for two FISH signals per AMCA-positive nucleated cell was 97%, comparable to the 98% ($z=0.74$; NS) obtained in control slides of male and female lymphocyte nuclei. Using the method in samples of pure first trimester fetal blood, it was found that the frequency of fetal erythroblasts expressing e-globin chains declines linearly between 7 to 14 weeks gestation ($y=-15.8x + 230.8$; $r^2 = 0.8$) and that 50% of fetal erythroblasts continue to express e-globin as late as 12 weeks. We conclude that this technique will improve the specificity and clinical utility of prenatal diagnosis using fetal erythroblasts from maternal blood.

Utilization of Fetal Skin Biopsy for Prenatal Confirmation of Trisomy 12 Mosaicism. *L.A. Flore¹, S.A. Ebrahim², H.M. Lee¹, R. Aatre¹, F. Qureshi², A. Johnson¹, M.I. Evans^{1,2,3}.* 1) Obstetrics and Gynecology, Hutzel Hospital/Wayne State, Detroit, MI; 2) Pathology; 3) Center for Molecular Medicine and Genetics.

Mosaic trisomy 12 has been reported both prenatally and in liveborns. Prenatal detection of such mosaicism poses a counseling dilemma as it is not immediately known if the abnormal cells represent the fetus or are of extra-embryonic origin. In most cases, cordocentesis is offered after a mosaic amnio result. However trisomy 12 cells generally cannot be isolated in blood, but have been identified in other tissue sources. A 36 year old G1 P0 woman underwent amniocentesis for advanced maternal age. Cytogenetic analysis revealed 47,XX,+12/46,XX. Initial ultrasound revealed an echogenic cardiac focus but otherwise normal fetal anatomy. The patient was referred to us for further investigation. Ultrasound evaluation in our center revealed prominent ventricles and left sandal toe. The cerebellum appeared smaller than expected for gestational age, and heart circumference appeared at lower limits of normal. Under ultrasound guidance, a fetal skin biopsy was performed at 20 weeks gestation, as well as a CVS. Fetal blood was obtained at the time of termination. Chromosome and FISH analysis were performed and the following percentages of trisomy 12 cells were found: cultured skin fibroblast 22%, cultured placental tissue 25%, and interphase blood 0.6%. The pregnancy was terminated at 23 weeks gestation. Post-mortem evaluation revealed dysmorphic features including posteriorly rotated ears with prominent folding of the left helix, hypoplastic nares, hyperterlorism, broad and splayed hallices, and prominent clitoris with poorly formed labia. Examination of the central nervous system revealed deep white matter microinfarct, heterotopias in the cerebellar white matter, and a subarachnoid congestion with focal hemorrhage. Our data demonstrate the usefulness of fetal skin biopsy in providing patients with further information regarding fetal status after prenatal chromosome analysis reveals trisomy 12 mosaicism. Fetal skin biopsy should be included as part of the evaluation, when true mosaicism is found in the amniotic fluid, particularly for those situations in which blood is uninformative.

Prenatal diagnosis of Jacobsen syndrome. *B.R. DuPont¹, G.A. Stapleton¹, S. Chapman²*. 1) Greenwood Genetics Ctr, Greenwood, SC; 2) Center for Women's Medicine, Greenville Memorial Hospital System Greenville, SC.

Jacobsen syndrome is a contiguous gene deletion syndrome caused by the deletion of chromosome 11q23 to qter. Persons with this deletion exhibit a variety of characteristics, including moderate to severe psychomotor retardation, trigonocephaly, heart defects, hypertelorism, low-set dysmorphic ears, and small stature. Prenatal diagnosis of Jacobsen syndrome has been previously reported for only a single case (McClelland et al., 1998). In this case report, the only abnormality detected at ultrasound was nuchal thickening. Examination after birth revealed other findings consistent with Jacobsen syndrome.

Our patient, a 37 year old G3P2 was referred at 22 weeks gestation for a genetic amniocentesis because of advanced maternal age and increased nuchal thickness on routine ultrasound. Targeted ultrasound at amniocentesis revealed multiple congenital anomalies including nuchal thickening from the base of the skull to the upper thoracic spine, a hypoplastic left heart, an irregularity of the lumbosacral area of the spine, bilateral clubbed feet, a widened iliac angle, and low-set ears. Fetal echocardiogram confirmed the heart defect. FISH results for aneuploidy were normal. The complete karyotype of the fetus revealed a terminal deletion of chromosome 11q23. The pregnancy was terminated at 23 weeks. The family declined postmortem examination. The parents' karyotypes were normal and neither expressed the folate-sensitive fragile site found at 11q23.3, FRA11B. To our knowledge, prenatal diagnosis of Jacobsen syndrome has previously been reported in only one other case.

Prenatal diagnosis of Limb Body Wall Complex: Role of fetal ultrasound and MR, correlation with phenotype, karyotype and autopsy findings. *M.A. Aguirre¹, G. Castineyra²*. 1) Dept Genetica Medica, Centro Nac Genetica Medica, Buenos Aires, Argentina; 2) Fundacin cientfica del sur, Buenos Aires, argentina.

The underlying mechanism of body wall closure remains a poorly understood process and may be control by homeobox genes. We reported a prenataly diagnosed male fetus with LBW complex. It was the first pregnancy of non consanguinious parents ;age 31 and 29 years. No teratogenic effects were detectable. A fetal ultrasound done at 16 weeks of gestacional age showed encephalocele and omphalocele. The ecography at 18 weeks by femoral longitud revealed encephalocele, meningomyelocele, anomalies of the thoracic cage, marked lordosis, gastroschisis and deformities of the limbs. At 22 weeks 2D scan was done with Doppler and Angio Power, abnormalities of vascularitation of abdominal organs and ventricular defects were found. MR at 24 weeks give evidence of parietal encephalocele and abdominoschisis. Gestacion was deliverity by cesarean section because fetal demise. Karyotype of the fetus was normal, 46XY. The autopsy findings were: microcephaly, parietal encephalocele attached to the frontal region with an anmiotic band, disruption of frontonasal process, probosis, bilateral cleft lip and palate, anterolateral body wall defect thoraco-abdominoschisis, evisceration of thorax and abdominal organs with marked hypoplasia, severe lordosis and scoliosis, neural tube like defect at lumbar region and limbs defect (upper left limb hypoplastic anmiotic band constriction of two digits of the right hand and genu recurvatum of the legs) and single umbilical artery. Radiographic showed gross lordosis, scoliosis ribon like ribs, marked hypoplastic bones of the left upper limb. Final diagnosis was LBW complex and differential diagnosis with other entities were defined. Our case highlight the importance of ultrasound in the diagnosis of fetal malformation, guide by a multidisciplinary team for major understanding in developmental pathology and fetal prognosis and parental managment.

Detection of Y chromosome material in prenatally diagnosed 45,X cases. *B. Huang¹, S. Bhatt¹, C.J. Sandlin¹, P.R. Pearle², D. Main², M. Thangavelu¹.* 1) Genzyme Genetics, Orange, CA; 2) California Pacific Medical Center, San Francisco.

Mosaicism involving a 45,X cell line is relatively common in prenatal diagnosis. The prognosis of prenatally diagnosed non-mosaic 45,X cases and 45,X mosaicism with a Y chromosome bearing cell line is different. Studies have found that over 90% of the prenatally diagnosed 45,X/46,XY mosaic cases resulted in grossly normal male phenotype. Therefore, accurate identification of the Y-bearing cell line is critical for appropriate genetic counseling and postnatal management. We present our experience with prenatally diagnosed cases with a 45,X cell line.

A total of 74 cases were found to have a 45,X karyotype by standard cytogenetic analysis. Of these, sixty-eight patients had abnormal ultrasound findings suggestive of Turner syndrome. Of the six cases with normal ultrasound findings, follow-up ultrasound examination identified male genitalia in three cases. Further cytogenetic and FISH analysis detected Y chromosome material in all three cases, one with a dicentric Y;14 chromosome and the other two having mosaicism with a marker chromosome containing the SRY locus. Our study demonstrated that most (68/74 or 91.9%) prenatally diagnosed non-mosaic 45,X cases have ultrasound findings suggestive of Turner Syndrome. The presence of Y chromosome material was identified in a significant portion (3/6 or 50%) of the cases without ultrasound abnormalities. This data suggests the need for further investigation in 45,X cases with normal ultrasound findings by performing follow-up ultrasound examination to confirm the fetal sex and/or increasing the cell/colony counts in cytogenetic analysis, which would enhance the ability to detect mosaicism.

A study of the attitudes of individuals affected with and at-risk for Hereditary Hemorrhagic Telangiectasia towards prenatal diagnosis. *J. Murphy*^{1,2}, *M. Faughnan*³, *R. Hyland*³, *S. Kennedy*¹. 1) Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, Canada; 2) Department of Molecular and Medical Genetics, The University of Toronto, Toronto, Canada; 3) Respiratory Medicine, St. Michael's Hospital, Toronto, Canada.

HHT is an autosomal dominant, genetically heterogeneous disorder involving abnormalities in the vascular structure. In 1994 and 1996, two causative genes for HHT were identified. HHT type 1 (HHT1) is on chromosome 9q34 and encodes the endoglin protein. HHT type 2 (HHT2) maps to chromosome 12q13 and encodes a TGF- β receptor, activin receptor-like kinase (ALK1). An endoglin protein assay and molecular testing, for both endoglin and ALK1, are currently available on a limited basis through a research laboratory. It is anticipated that molecular testing will eventually become available on a clinical basis and that prenatal diagnosis (PND) will become an option for families with HHT. We surveyed the attitudes of affected and at-risk individuals with HHT towards PND to assess the potential demand for this service. From two centres in Toronto, a total of 207 eligible individuals were invited to participate in the study. Preliminary results from 53 completed surveys indicated that the majority are interested in utilizing PND. Demographic factors, other than sex, did not appear to impact perceived utilization of this service. Most respondents indicated that they would want to have a say in whether they received PND for HHT, and the majority indicated that they would only use this service if it had a 0% risk of miscarriage. Perceived burden of disease and the severity of symptoms of HHT were found to be predictive factors of an individual's willingness to use PND that has a risk of miscarriage. These factors also appeared to impact upon the acceptability of termination of pregnancy for HHT, although the vast majority felt that termination of pregnancy for HHT was unacceptable. By gaining a better understanding of the factors involved in decision making regarding PND for HHT and patient interest in this service prior to its availability, we hope to be better prepared to serve and understand the needs of this patient population.

Prenatal diagnosis of trisomy 20: resolving the 20 year controversy. *N.B. Kardon¹, J. Marks², G. Lee¹, T. Dunn¹.* 1) Department of Human Genetics and Pediatrics, Mount Sinai Medical Ctr, New York NY; 2) Department of Obstetrics and Gynecology, Lenox Hill Hospital, New York NY.

The clinical significance of mosaic and non-mosaic trisomy 20 detected prenatally needs careful consideration and evaluation on a case by case basis. We report a case in which the initial chromosome analysis revealed a non-mosaic trisomy 20 or 47,XY,+20. The patient had been referred for amniocentesis due to advanced maternal age. The ultrasound findings of the fetus were normal as were the AFP values. At the time of the amniocentesis a collapsing structure next to the fetus was noted. Upon careful consideration a repeat amniocentesis and high level ultrasound were performed. The second sample of amniotic fluid was taken on the side opposite from the collapsing structure believed to be an extra-embryonic sac. The second analysis revealed a normal karyotype in 90% of the cells and trisomy 20 in the remaining 10%. The pregnancy continued to term. The infant appeared normal at birth and is developing normally. Several cell lines were analyzed at this time, all of which demonstrated a normal 46,XY chromosome complement. The trisomy 20 cells in the two amniotic specimens were derived from the extra-embryonic sac believed to be a vanishing twin. In conclusion careful consideration must be taken before any risk assessments are offered to patients with prenatal trisomy 20 results. The value of repeat amniocentesis and careful ultrasound should not be overlooked. The majority of these cases do not represent the true chromosomal complement of the fetus.

A new method using lectin for isolation of fetal cells from maternal blood. *M. Kitagawa^{1,2}, K. Sugiura², T. Okuyama³, K. Kanayama¹, M. Natori¹, D. Wakamatsu⁴, H. Yura⁵, H. Sago^{1,2}.* 1) OB&GYN, Natl. Okura Hosp, Tokyo, Japan; 2) Jikei Univ. Tokyo, Japan; 3) Natl. Children's Med. Res. Center, Tokyo, Japan; 4) Kawasumi Lab. Inc., Tokyo, Japan; 5) Natech Inc., Japan.

To isolate fetal cells from maternal blood, we developed a new method which used lectin, a kind of protein, specifically recognizes sugar chains expressed on surface of blood cells. This method utilized difference in affinity to lectin between different blood cells. Nucleated red blood cells (NRBCs) were selectively attached to a substrate coated with a sugar-containing high-molecular-weight material (PV-MeA) via soybean agglutinin, a lectin. Cord blood samples were used to evaluate enrichment efficacy of NRBCs. Using this method, we isolated one to several hundreds of NRBCs in 7 ml peripheral blood samples from 96% of 113 normal pregnant women between 6 to 27 gestational weeks. The isolated NRBCs were analyzed by FISH with probe for Y-chromosome in eight cases carrying male fetuses. More than half of NRBCs were of fetal origin. The study demonstrates that our new method using lectin provides effective enrichment of fetal NRBCs allowing noninvasive prenatal cytogenetic and molecular analysis.

DNA Prenatal Diagnosis of Citrullinemia: A Case Report. *K.M. Hong¹, M.K. Paik¹, S.H. Hahn²*. 1) Department of Biochemistry, Wonkwang University College of Medicine, Iksan, 570-749, Korea; 2) Department of Pediatrics, Ajou University School of Medicine, Suwon, 442-749, Korea.

DNA prenatal diagnosis was successfully performed on a family with citrullinemia. The father had a G324S mutation and the mother had an IVS6-2A>G mutation in argininosuccinate synthase (ASS) gene. They had a previous child with citrullinemia who died in a week after birth due to complicated hyperammonemia. Subsequent DNA study showed that the lost child was a compound heterozygote. DNA was extracted from cultured amniotic cells at 18-week gestation of this pregnancy. For a detection of the G324S mutation, PCR-RFLP method was used, and for IVS6-2A>G mutation, allele-specific PCR was performed. The fetus was found to have G324S mutation but not IVS6-2A>G mutation suggesting a heterozygote. Pregnancy was continued and a healthy boy was born at 39 weeks of gestation with birth weight of 3.59 kg. Plasma amino acid analysis performed on the third day after birth was normal and serial ammonia level was in the normal range. Molecular study on his genomic DNA was also coincided with his previous fetal DNA analysis. He is now 2 months old growing normally.

SURVEY OF MATERNAL SERUM SCREENING PRACTICES IN THE GREAT LAKES REGION. T.W.

Kurczynski¹, M.A. Gray¹, R.A. Fisher², M.E. Hodes³, J.L. Williams⁴. 1) Mercy Children's Hospital, Medical College of Ohio, Toledo, OH; 2) Dept Pediatrics/Human Development Michigan State University, East Lansing, MI; 3) Dept Medical & Molecular Genetics Indiana University School of Medicine, Indianapolis, IN; 4) La Crosse Regional Genetics Services La Crosse, WI.

The Screening Issues Subcommittee of the Great Lakes Regional Genetics Group completed a survey of current practices in maternal serum screening in the Great Lakes region. Questionnaires were sent to genetic counselors involved in prenatal counseling identified from the National Society of Genetic Counselors. Prenatal screening laboratories identified by counselors were sent questionnaires with follow-up telephone contact. Thirty-one maternal serum screening laboratories were identified. Twenty-four (77%) completed the laboratory questionnaires. Most of the counselors were not involved with laboratory procedures but interacted with multiple laboratories. The laboratories showed considerable variation in the following features: type of human chorionic gonadotropin(HCG) assay; multiple of the median(MOM) cutoff for open structural defects and Down syndrome; use of ultrasound dating; handling discrepancies between gestational age based on ultrasound and last menstrual period; method of adjustment for race, insulin dependant diabetes, and weight; MOM cutoff for multiple gestations and increased Down syndrome and neural tube defect risks; handling of missing information on the requisition; use of information from a positive family history in assessing risk; reporting of term or midtrimester risk; use of published or in house detection rates and false positive rates; frequency of calculation of medians and monitoring for assay drift. Most laboratories had limited information on pregnancy outcome and epidemiology of the client population. All of the laboratories participated in a quality assurance program and all but two were College of American Pathology(CAP) certified. This survey demonstrates considerable variation in laboratory procedures in prenatal maternal serum screening in the Great Lakes region. This variation can influence the assessment of prenatal risks.

Residual risk of chromosome abnormality in prenatal diagnosis after a normal interphase FISH result. J.P.

Homer, S. Bhatt, B. Huang, M. Thangavelu. Dept Clinical Genetics, Genzyme Genetics, Orange, CA.

Aneuploidy involving chromosomes 21, 13, 18 and the sex chromosomes constitutes the majority of abnormalities observed during prenatal diagnosis. Advent of interphase FISH in prenatal diagnosis has allowed for detection of the most common chromosome abnormalities within 24-48 hours. Normal FISH results can provide reassurance to worried patients and relieve anxiety to a large extent. The question remains as to what is the likelihood of a chromosome abnormality in view of a normal FISH result, i.e., what is the residual risk? We have analyzed our data on over 21,000 amniotic fluid and chorionic villi samples received for prenatal diagnosis for years 1998 and 1999, to determine the usefulness of FISH in the detection of chromosomal abnormalities. The clinical indications included for the study are advanced maternal age (AMA), abnormal maternal serum screen indicating an increased risk for a chromosomal abnormality (CHAB) or neural tube defect (NTD) and ultrasound abnormalities (US) which constitute the most common reasons for prenatal diagnosis in most laboratories. The proportion of abnormalities not detected by FISH alone including structural abnormalities (unbalanced, balanced, de novo as well as inherited), marker chromosomes, aneuploidies involving other chromosomes and mosaics was 34.4%. The proportions within each category were: AMA-33.2%, CHAB-42%, NTD-100%, US-26.3%. The overall residual risk for an abnormal chromosome result after a normal FISH was 1.2 %. The risk in each category was AMA-1.1 % , CHAB-1.7%, NTD-1.3% and US-2.9%. These data emphasize the need for caution in interpreting FISH results in prenatal diagnosis and the importance of offering interphase FISH only in conjunction with conventional cytogenetic analysis. It also provides information regarding residual risk that will be useful to genetic counselors to use in their sessions with the patients.

Reliable detection of extra-cellular fetal DNA in maternal blood. *D.N. Saller Jr¹, C. Wei², J.W.H. Sutherland²*. 1) OB/GYN, Div Maternal-Fetal Med, Strong Memorial Hosp, Rochester, NY; 2) Ortho-Clinical Diagnostics, Johnson and Johnson Corporation, Rochester, NY.

Following the observations of Lo et al (Am J Hum Genet 1998, 62:768-775), we hypothesized that extra-cellular fetal DNA could be reliably detected and quantitated in maternal blood. Thirty patients had 10 cc of blood drawn prior to clinically indicated CVS or amniocentesis. DNA was isolated and concentrated using a commercial kit (QIAamp DNA Blood Midi Kit, Quiagen Corporation, Valencia, CA). b-actin and the SRY gene were quantitated by real-time PCR monitoring (TaqMan PCR Reagent Kit, Perkin-Elmer Corporation, Alameda CA). Of the 30 patients, 19 were carrying a male fetus and 11 were carrying a female fetus. In all 30 cases, the prediction of fetal gender was correct. Of the two cases with the greatest amount of fetal DNA, one of them was noted at amniocentesis to be a case of fetal trisomy 21. Our data suggest that extra-cellular fetal DNA can be reliably detected and quantitated when present in maternal blood. Additionally we speculate that if the observation of an increased amount of extra-cellular fetal DNA in maternal blood in pregnancies complicated by fetal aneuploidy is confirmed in larger data sets, it may have direct application to maternal serum screening for fetal Down syndrome or other aneuploidies.

Prenatal Diagnosis of Apert Syndrome - Report of Two Cases. A. Pai¹, A. Toi¹, G. Seaward¹, L. Steele², D.

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Apert syndrome is an autosomal dominant condition, accounting for 4.5% of cases with craniosynostosis. The clinical manifestations include brachycephaly, midfacial hypoplasia, proptosis, broad thumbs and great toes and symmetric syndactyly of the fingers and toes typically presenting as mitten like hands and feet. Most cases are sporadic and result from a mutation in the paternal FGFR2 gene. Two mutations (S252W and P253R) account for 99% of cases of Apert syndrome. Prenatal diagnosis of Apert syndrome has been described in eight cases. In seven of those, a diagnosis was made between 26 and 34 weeks. In one case, diagnosis was made at 18 weeks and 6 days gestation. We present two cases with Apert syndrome diagnosed prenatally. Case 1: A routine ultrasound on a patient at 17 weeks 5 days gestation demonstrated mitten hand syndactyly and mildly dilated ventricles. Further ultrasonography at 19 weeks and 6 days gestation revealed cloverleaf skull (Kleeblattschadel), mitten hands, proptosis and low set ears. A diagnosis of Apert syndrome was confirmed by DNA analysis, which showed a C767G (S252W) mutation. Autopsy results confirmed Kleeblattschadel, large fontanelles, midface hypoplasia, shallow orbits, narrowing of the palate, syndactyly of hands and feet and coarctation of aorta. Case 2: An ultrasound at 21 weeks gestation demonstrated hypoplastic left heart syndrome, Kleeblattschadel, and dilated cerebral ventricles. Post-termination syndactyly of the fingers and toes were detected, and a diagnosis of Apert syndrome was made. This was confirmed by DNA analysis, which showed a C767G (S252W) mutation. The above cases show the importance of detailed prenatal ultrasound in combination with DNA analysis in making a diagnosis of Apert syndrome in cases diagnosed prenatally with Kleeblattschadel. Congenital heart defects, mainly left sided, have been reported in 10-23% of cases of Apert syndrome and should raise suspicion and help in making this diagnosis.

Rapid prenatal detection of Down and Edwards syndromes by fluorescent polymerase chain reaction with STR markers. *H.R. Yoon¹, Y.S. Park¹, A.N. Lee¹, S.H. Kim².* 1) Section of Cytogenetics, Seoul Clinical Laboratory, Seoul Medical Science Institute, Seoul, Korea; 2) Section of Cytogenetics, Samsung Medical Center, Sungkyunkwan University, Seoul, Korea.

Prenatal diagnosis for trisomies is routinely performed using chromosome analysis of fetal cells. Although chromosome analysis allows accurate diagnosis, it should follow successful in vitro cultures, which require great technical expertise and time-consuming manual procedures. Recently, alternative methods, which provide rapidity and accuracy without culture, have been developed to be applied particularly to cases of pregnant women requiring rapid decision for termination. The aims of this investigation were to evaluate the clinical usefulness of fluorescent polymerase chain reaction (F-PCR) for rapid prenatal detection of Down and Edwards syndromes and then to construct the basic data for clinical application. DNA were extracted from amniotic fluid, peripheral blood and archived slides derived from 47 normal karyotype, 23 Down syndrome and 8 Edwards syndrome subjects. Multiplex F-PCR were performed by concurrent use of short tandem repeat (STR) D18S535, D18S51, D21S11 and D21S1412 markers specific for chromosome 18 or 21. Electrophoretic analysis and calculation of fluorescent intensities of PCR products were performed by ABI 310 genetic analyzer. Normal samples displayed diallelic peaks for each STR marker. Reference ranges of ratio of peak areas calculated were 1.0-1.4 for D21S11, 1.0-1.5 for D21S1412 and 1.0-1.3 for D18S535 and D18S51. Down and Edwards syndromes showed characteristic triallelic peaks of similar intensity corresponding to 3 different alleles or characteristic diallelic peaks with ranges of peak area ratio being 1.5-3.4. F-PCR allowed simultaneous prenatal diagnosis of Down and Edwards syndromes to be performed within 8 hours using amniotic fluid. The sensitivity, specificity and efficiency of the test for detection of Down and Edwards syndromes were 96.7%, 93.6% and 94.8%, respectively. In conclusion, these results provide clear evidence that F-PCR detect Down and Edwards syndromes more rapidly with high accuracy and normal reference ranges for clinical application of F-PCR.

46,XX/46,XY karyotype in a phenotypically female abortus. *F.R. Parikh, P.F. Madon, A.S. Athalye, R.P. Soonawala.*
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A 28 year old woman had a preterm induced breech vaginal delivery in the 8th month of pregnancy. There was history of a first trimester spontaneous abortion in the first pregnancy and a Caesarian section with fetal distress in the second pregnancy. The abortus was a fresh, 1.85kg. stillborn female. The liquor was meconium stained. The indication for abortion was severe intrauterine growth retardation, severe oligohydramnios and a strong possibility of diaphragmatic hernia diagnosed by ultrasonography. The dysmorphic features noted on delivery were upward slanting eyes, a bulbous nose, elongated philtrum, large low set ears and a carp-shaped mouth. There was loose skin on the nape of the neck, a hairy back, and a single palmar crease on the left hand. The external genitalia were typically female. An autopsy was not permitted.

Karyotyping of GTG-banded metaphases from cord and placental blood showed a 46,XX/46,XY mosaic pattern in the 45 metaphases studied, the XY cell line being present in two-thirds of the cells. Quinacrine fluorescence confirmed the Y chromosome. No other numerical or structural chromosome aberrations were detected. Most of the cases of XX/XY mosaicism detected prenatally, had a male fetus. The present case with a phenotypically female fetus could indicate a vanishing twin, maternal cell contamination in a case of testicular feminization or a true hermaphrodite. Histopathology of fetal gonads would ideally have lead to a precise diagnosis.

Prenatal detection of monosomy 13 and X mosaicism is shown to be a twin demise by microsatellite analysis. K.K.

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We report a case of apparent true mosaicism for a cell line with both monosomy 13 and monosomy X and a normal 46,XY cell line detected in an otherwise routine prenatal diagnosis study. Two colonies from the primary culture, one colony each from the second and third cultures, and four cells from subcultures had a 44,X,-13 chromosome complement. The indication for amniocentesis was advanced maternal age (39 yr) with a positive triple screen for Down syndrome (1 in 78 risk). Ultrasound examination by a perinatologist at the time of amniocentesis (19.3 wk) and under high resolution at 23 weeks showed a normal male fetus. PUBS was not recommended. Comparison of chromosome polymorphisms between the cell lines showed no obvious differences. Microsatellite analysis was performed to clarify whether an undetected twin demise may have contributed to the abnormal cell line. DNA was extracted from the amniocytes, and seven random loci were amplified by PCR. Gel analysis revealed 3 alleles at four of the loci, and 1, 2, and 4 alleles each at the other three loci. The presence of more than 2 alleles at any of the loci confirmed that the DNA came from more than one fetus. These data underscore the importance of using molecular analyses to clarify unusual prenatal cytogenetics results.

Early Prenatal Diagnosis of at risk case with Walker-Warburg syndrome. *M. Thomas, A. Pai, A. Toi, M. Sgro, T. Barozzino, D. Chitayat.* Prenatal Diagnosis, Medical Genetics and Pediatrics Program, Mount Sinai Hospital, Toronto, Ontario, Canada.

Walker-Warburg syndrome is an autosomal recessive consistent with eye, brain, muscle as well as other visceral abnormalities. We report early prenatal diagnosis of WWS and results of ultrasound follow-up in a fetus at risk. The couple was East Indian and non-consanguineous. Their first pregnancy resulted in a healthy daughter. The second pregnancy resulted in a daughter with WWS who died at 9 months. In the third and fourth pregnancies, affected fetuses were detected by ultrasound and the pregnancies were terminated. Their recent pregnancy was followed from 8.7 weeks gestation with repeat fetal ultrasound. At 14 weeks gestation fetal ultrasound was reported as normal. Fetal ultrasound at 18 weeks revealed lateral ventriculomegaly (14 mm), prominent 4th ventricle, and hypoplastic vermis. By 21 weeks gestation, there has been further ventricular enlargement (17 mm), and the cerebellum was at the 25th percentile. Partial vermian agenesis was also evident. At 23 weeks gestation, the lateral ventricles measured 18 mm. The couple decided to continue the pregnancy and a male was born at term. His birth weight was 4.2 kg (97th centile) and OFC 37 cm (97th centile). He was noted to be hypotonic with a myopathic facies and wasting of the muscles of the thenar and hypothenar eminences. Dilated eye exam showed retinal dysplasia. On day 4 of life the blood CK level was 3180 IU/L (normal <390). He developed progressive hydrocephalus with a peak head circumference of 46 cm on day 14 before a VP shunt was placed. He had temperature instability with episodes of hyper and hypothermia of central origin. WWS is an autosomal recessive multisystemic disorder affected the cerebrum, cerebellum, eyes, striated muscles as well as the urogenital system. The prenatal progression of the condition and thus the stage at which at risk fetus can be detected has not been delineated. This case shows that the ventriculomegaly progresses slowly and while at 18 weeks gestation it measured 14 mm at 21 weeks it reached 17 mm. The cerebellar hypoplasia and the partial vermian agenesis were noted at an early stage.

Prenatal Counselling, Investigative Options and Outcomes Following Fetal Ultrasound Finding of Echogenic

Bowel. *S.J. Withers¹, L. Steele², P. Ray², E. Winsor³, L. Velsher¹, M. Thomas¹, A. Pai¹, D. Myles¹, D. Cushing¹, S. Conacher¹, A. Toi⁴, D. Chitayat¹.* 1) Prenatal Diagnosis Program, Toronto General Hospital, Toronto, Ontario, Canada; 2) Molecular Genetics Laboratory, Hospital for Sick Children, Toronto, Ontario; 3) Department of Cytogenetics, Toronto General Hospital, Toronto, Ontario; 4) Department of Diagnostic Imaging, Mt. Sinai Hospital, Toronto, Ontario.

The finding of echogenic bowel on prenatal ultrasound has led to the establishment of noninvasive and invasive protocols to investigate the possible causes of this finding. We describe a prospectively collected series of 228 patients referred to the Prenatal Diagnosis Program over 11 years. Echogenic bowel was identified on ultrasound between 14 and 32 weeks (average 18.6). 105 (46%) had echogenic bowel as the only finding at presentation. 24 (10%) had echogenic bowel and a positive maternal serum screen as their only findings. Average maternal age was 31.1 years. The counselling provided following referral was that the echogenic bowel may represent 1. A benign condition, 2. A chromosomal disorder, 3. Cystic Fibrosis or 4. Maternal Infection. All women were offered the options available to investigate the potential cause of the echogenic bowel. The non-invasive options offered were parental cystic fibrosis mutation testing and maternal STORCH serology. Invasive testing offered was amniocentesis. Two couples reported a family history of cystic fibrosis. 118 elected to have amniocentesis performed (52%). Of these, 17 karyotypes were abnormal (7%). 183 women (80%) and 178 partners (78%) underwent CF testing. Screening for 4 mutations, 10 and later 31 mutations were screened for as they became available. Two couples were carriers of CF mutations. One affected fetus was identified. In total 13 parents were ascertained as carriers of CF mutations (13/361, 3.6%). No cases of STORCH infections were identified. Echogenic bowel is an important sign in prenatal diagnosis. In approximately 1 in 200 cases it indicates fetal cystic fibrosis. In a fetus with multiple congenital anomalies including echogenic bowel the risk for having a chromosome abnormality is high.

A prospective evaluation of several approaches to the first trimester screening for aneuploidies. G.L.

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The pilot program including ultrasound examination of 35,942 first trimester pregnant women with retrospective biochemical testing of 15,000 serum samples for AFP, Free b-hCG and partly for PAPP-A was carried out. Based on the predictions collected from the pilot program, we started a prospective comparative study of several approaches to the first trimester screening for aneuploidies. Three protocols for selection of pregnant women for fetal karyotyping are compared: US - the cut-off of the fetal nuchal translucency thickness ³ 3 mm.; USR - the risk of the fetus having trisomy 21 was estimated by a combination of maternal age and fetal nuchal translucency thickness (NT); CSR - the risk of the fetus having trisomy 21 was estimated by a combination of maternal age, NT and biochemical markers (AFP, PAPP-A, free b-hCG). The risk of 1:360 at the expected date of delivery was chosen as the cut-off for an invasive procedure. By May 1, 2000, three groups (5,887; 5,420 and 4,384 respectively) of unselected pregnant women were tested according to the protocols. Maternal age distribution (range 16 - 44) was the same in each group, including 6.4% of women of 35 and older. 93% of pregnant women were screened at CRL of the fetus between 38 and 70 mm. (range 38-78). 3-US, 4-USR and 6-CSR cases of trisomy-21 were detected with the expected numbers of 6.8, 6.8 and 5.03, respectively. Moreover, it appears to be that 90% of trisomies-13 and -18 as well as X monosomy can be revealed. The study of birth incidence of aneuploidies in the groups screened is now in progress.

Autosomal Dominant Omodysplasia: Prenatal Diagnosis. *C.P. Venditti¹, J. Farmer², C. Friedrich², M. Mennuti³, D. Driscoll^{3,1}, L. Whitaker⁴, E.H. Zackai¹.* 1) Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia; 2) Department of Medical Genetics; 3) Department of Obstetrics and Gynecology; 4) Department of Plastic and Reconstructive Surgery, Hospital of the University of Pennsylvania, Philadelphia, PA.

Robinow's original report in 1969 described an AD syndrome with mesomelic brachymelic dwarfing. However, the majority of cases since reported have been sporadic or AR. In fact, one of the AD examples reported in 1982 by VallJe and Maroteaux has been reassessed and described as omodysplasia. This syndrome is distinguished from Robinow syndrome in having humeral shortening as opposed to mesomelic disproportion and characteristic abnormal elbows. Our patient was seen in early childhood because of short arms, widely-spaced eyes and hypoplastic genitalia and carried the diagnosis of Robinow syndrome. She was referred at age 25 during her first pregnancy after an early ultrasound revealed absent humeri of the fetus. Her previous x-ray findings were reviewed and revealed shortened humeri with abnormal sloping of the distal articular surface, shallow olecranon fossae with partially subluxed radii, short first metacarpals, and small laterally displaced patellae. She was then diagnosed with omodysplasia. The 15 week ultrasound showed a space between the shoulder and elbow without ossification of the humerus and a bossed skull. It was at this time that we suspected the fetus as being similarly affected as the patient and the pregnancy was followed. We suggested that this was the 2nd known example of AD omodysplasia. A repeat ultrasound at 20 weeks showed a normal tibia and fibula, mild shortening of the radius, ulna, and femora with ossified but markedly shortened humeri. Genitalia were visualized but not assignable to either sex. No other abnormalities were appreciated. This report illustrates that individuals who carry the diagnosis of Robinow syndrome should be evaluated as to whether the shortening is mesomelic or rhizomelic. The latter suggests the diagnosis of omodysplasia. The delayed ossification of the humeri in the fetus may provide insight into the pathophysiology of this disorder.

Effect of ethnicity and insurance status on the acceptance rate of invasive prenatal cytogenetic testing after a positive screening result. *J. Santolaya - Forgas, E.M. Powell, J.K.B. Matheson, L.P. Shulman.* Dept OB/GYN, Univ of Illinois- Chicago, Chicago, IL.

Prenatal screening is designed to detect women at greater risk of having children with congenital abnormalities. Maternal age greater than 35 at the time of delivery (MA), maternal serum triple markers (MSM) and ultrasound (US) are currently the most commonly used screening protocols. Amniocentesis or CVS are offered to women with positive screening to diagnose fetal chromosome abnormalities. We have previously reported a 37% overall acceptance rate for amniocentesis/ CVS after genetic counseling at our institution; during the years 1995-1999, of 1695 tests offered, 625 patients accepted further genetic testing (ACMG, 2000). In order to evaluate if ethnicity and insurance status are related to the acceptance rate of prenatal testing, we studied 700 pregnant women referred for genetic counseling from Jan 1999 to Jan 2000. 309 (44%) women were Hispanic, 271 (39%) were African American, 91 (13%) were Caucasian and 29 (4%) were of other ethnic backgrounds, mainly Asian. 197 (28%) had private insurance while 503 (72%) had no insurance or were on Medicaid. Of the 700 women, 159 (22.7%) were referred for other indications that did not require further invasive genetic testing. Of 541 women, 86 out of 146 (59%) insured patients underwent invasive genetic testing, while only 131 of 395 (33%) non-insured patients had further testing. Although the overall acceptance rate was similar for all indications in the insured group, there was significant variation in the non-insured group: 27% for MA, 34% for MSM and 58% for US. Differences were also noted based on ethnic background. Among the insured population, 72% of Caucasians, 53% of Hispanics and 44% of African Americans accepted further testing. In the non-insured group, 54% of Caucasians, 36% of Hispanics and 25% of African Americans pursued further testing. We are currently assessing if these results depend on information provided to the patient concerning the objectives of the prenatal screening program, are due to a lack of patient's interest in these objectives or are influenced by additional external patient factors.

Prenatal diagnosis of Saethre-Chotzen syndrome. *G.M. Cohn, V. Loik Ramey.* Clinical Genetics, Baystate Medical Ctr, Springfield, MA.

We present a family with two children and a pregnancy affected with Saethre-Chotzen syndrome. The first child was diagnosed postnatally on clinical exam, the second prenatally by ultrasonography, and the current pregnancy was diagnosed by mutational analysis of the TWIST gene through amniocentesis.

Our patient presented in a current pregnancy for prenatal diagnosis of Saethre-Chotzen syndrome. She had two children with clinical diagnoses. The patients husband was described as having a small head, but did not carry a formal diagnosis. The family was initially ascertained in the patients pregnancy for her daughter following a third trimester ultrasound that revealed microcephaly. At that time, it was noted that the patients son had physical features consistent with a craniosynostosis. The son was born in the Dominican Republic with no prenatal ultrasounds. He was noted at birth to have microcephaly and a very "hard skull" but was not given a diagnosis. A formal genetics evaluation revealed a diagnosis of Saethre-Chotzen. The daughter was born with similar physical features to her brother and was also given a diagnosis of Saethre-Chotzen. The patient then returned in her current pregnancy requesting prenatal diagnosis.

Prior to amniocentesis, genetic testing was performed on the patients son and daughter and revealed they both carried the Q112X mutation in the TWIST gene previously reported in individuals with Saethre-Chotzen syndrome. The patient had an amniocentesis with Saethre-Chotzen testing which revealed the same mutation in the fetus. Ultrasounds in the second trimester were unremarkable with normal cephalic measurements.

This case is unique in that it represents the first reported cases of prenatal ultrasound findings and prenatal diagnosis of Saethre-Chotzen syndrome. While ultrasonography was useful in suggesting a diagnosis in the second pregnancy, the absence of anomalies through the second trimester of pregnancy in a genetically affected fetus suggests that ultrasound alone may not be a reliable method of prenatal diagnosis for this condition.

Fetal Pathology of Ultrasound Diagnosed Fetal Abnormalities Comparing Surgical and Induction of Labor Terminations of Pregnancy. *R.D. Wilson^{1,2}, R.T.L. MacPherson², B. McGillivray², J.M. Friedman², D. McFadden³.*
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Objective: The purpose of this study was to examine the influence of the method of pregnancy termination on the ability to confirm prenatally diagnosed ultrasound abnormalities using the fetal pathology examination as the gold standard.

Study Design: This study involved a retrospective chart review of patients between Jan. 1, 1991 and Jan 1, 1996. Maternal, ultrasound, cytogenetic and autopsy information was available for 521 consecutive pregnancy terminations for fetal anomalies. These were compared on the basis of method of termination, results of prenatal cytogenetic investigations and gestational age at termination.

Results: The ability to evaluate specific ultrasound diagnosed fetal anomalies was less likely at autopsy following surgical compared to medical terminations (by odds ratio) depending on specific anomalies. Cystic hygroma 146.18 $p < 0.00001$; abdominal wall defects 30.60 $p = 0.00107$; structural heart defects 21.00 N.S.; diaphragm defects 19.00 N.S.; fetal hydrops 9.00 N.S.; anencephaly 8.91 N.S. Confirmation of CNS, heart and kidneys was more likely for each increased gestational week.

Conclusion: The trend in each case was for surgical termination to be informative less frequently than medical termination. For surgical terminations the ability to accurately evaluate CNS, heart and kidneys improved as gestational age increased.

Termination of pregnancies for fetal indication: 9-year experience. *R. Sharony, A. Amiel, S. Federman, J. Grinshpun-Cohen, M. Fejgin.* Genetic Institute, Sapir Medical Center-Meir Hospital, Kfar Saba, Israel.

Objective: To analyze the different reasons for termination of pregnancy and the breakdown of the demographic data and the stage in which they were done. Design: Descriptive study of the referrals for pregnancy termination at Meir General Hospital in Israel. In this country all the patients undergo triple screen between 16-20 weeks and ultrasonic scan to detect fetal abnormality at 20-22 weeks. However, many undergo also an early U/S scan. The attitude towards amniocentesis is very liberal and approximately 10% of the patients under age 35 elect to undergo amniocentesis. Participants: 353 women who were referred for pregnancy termination between the years 1989 and 1997. During those years there were about 50,000 deliveries in our center. Main outcome measures: The epidemiological data regarding the referrals to the committee: gestational age, maternal age, the fetal abnormality and the way it was picked up. Results: Most fetal karyotype results become known only around the 20th week. The average number of termination at our center was around 40 annually. Mean maternal age was 29 and mean gestational age at termination was 20 weeks. Most fetal indications fell into two categories: 144 (40 %) for fetal aneuploidy and 209 (60 %) following ultrasonic findings. The karyotype of 47, XX and 47, XY +21 was the most common chromosomal aberration (73-20%) followed by sex chromosome anomalies (19-5%). Neural tube defect was the most frequent ultrasonic diagnosed indication (55-16%) followed by cardiac defects (28-8%). No preponderance for maternal advanced age was detected. Conclusions: The sonographically detected abnormalities composed about two thirds of the indications while fetal chromosomal anomalies were only one third of the reason for referral. However, the increase in the utilization of an early scan in which genetic markers are sought, may expand the proportion of chromosomal indication for pregnancy termination.

A female patient with Xp22.1 deletion shows complete X chromosome inactivation skewing and multiple spontaneous abortions; further support for X-linked cell lethal genes. *C.A.G. Scacheri¹, J.M. Giron¹, N.R. Agan², B. Amin¹, E.P. Hoffman¹*. 1) Children's Research Institute, Washington, D.C; 2) Baylor College of Medicine, Houston, TX.

Lyonization occurs early in female embryogenesis and is usually random. Skewed X chromosome inactivation (XCI) may be the result of primary mutations in the XIST gene or may be secondary to one X chromosome harboring a "cell lethal" mutation and/or having a growth disadvantage.

Here we present a patient who underwent prenatal diagnosis for maternal age. A heterozygous Xp22.1 deletion was identified in her female fetus, and cytogenetic testing performed on the patient showed a 46,X,del(X)(p22.1).ish del(X)(STS-) karyotype. This confirmed maternal inheritance of the deletion. FISH studies determined hemizygosity of the steroid sulfatase gene, broadening the deleted region to include Xp22.3. The pregnancy outcome was a girl with Sturge-Weber syndrome who died at 6 months. Prenatal dx in the patient's current pregnancy identified a 46,XX karyotype.

Our laboratory performed XCI studies on the proband, her two living daughters, her sister and her mother using PCR. Individuals with the Xp22.1 deletion were identified in all 3 generations and each showed completely skewed XCI (100%:0%). Our findings demonstrate the heritability of the deletion as well as its association with cell lethality and complete XCI skewing in this family.

To further delineate the deletion and identify the responsible X-linked lethal gene(s) throughout Xp, we mapped the deletion to a 36 kb region. We hypothesize that this region contains one or more genes that, when deleted or mutated, are not only lethal to individual cells in females but also cause prenatal death to males inheriting the mutation. Such a model of X linked cell lethal genes would be responsible for recurrent spontaneous abortion in females with nonrandom XCI patterns.

Progesterone and LHRH agonist retards physiologic ovarian follicle loss in rhesus monkeys: A Possible method for decreasing the rate of oocyte non-disjunction. *K.M. Ataya.* CASE WESTERN RES U/ MetroHealth Medical Center, Dept. Ob/Gyn, Cleveland, OH.

Recent evidence suggests that LHRH agonists and progestins may delay the progressive physiologic depletion of ovarian follicles in rats. LHRH agonists and progestins have also been reported to prevent chemotherapy induced ovarian toxicity in rats and rhesus monkeys and women. We investigated the effects of LHRHa (Leuprolide depot) combined with progesterone on the rate of loss primordial/ primary ovarian follicles in rhesus monkeys. Adult menstruating monkeys received subcutaneous progesterone interscapular implants to result in a progesterone concentration of >10 ng/ml, in combination with Lupron depot given as monthly intramuscular injection to release 50 ug/day. The control group was sham operated and received 0.9%NaCl injections. The first ovary was removed in each monkey before the initiation of treatments in all groups. Body weight, serum progesterone, estradiol, were serially measured. At the end of treatment, the second ovary in each monkey was removed. Both ovaries were serially sectioned. The number of primordial/primary follicles was counted in every 100th section. For each monkey, the follicle count in the second ovary was subtracted from the follicle count of the first ovary. The difference in follicle counts was then compared between the groups. The data is presented as mean + SD: * Control LHRHa+Progesterone p Monkeys # 53 Prim. Follicle Loss/Day 5.0+2.2 *0.97+0.54 0.006 The results indicate, that in rhesus monkeys, Progesterone combined with LHRHa significantly reduced the rate of loss of ovarian follicles compared to control. These findings may have important implications relating to delaying menopause and possibly decreasing the rate of meiotic non-disjunction in oocytes in the peri-menopausal time period.

Prenatal diagnosis of wide fetal abdominal wall defect. Problems in differential diagnosis and genetic counseling.

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An unusual number of cases of fetuses with wide abdominal wall defect were referred to our centers during 1999. We present four cases and asses the role of different imaging techniques in the diagnosis and prognosis of this condition and its possible associated features. We performed 2D ultrasound scans, 3D and MRI at different gestation ages. We highlight the syndromes with mayor abdominal wall defect different from omphalocele and gastroschisis whose prognosis varies completely. These included the Limb-body wall complex, pentalogy of Cantrell, Body stalk anomaly, OEIS and anmiotic band sequence. These syndromes also envolved skeletal anomalies absence or malformation of the limbs, cleft lip and thorachoschisis with ectopia cordis and are always lethal at birth. In our experience current 2D ultrasound showed to be the best method not only to ascertain the diagnosis but also in the follow up. We emphasise the need of a thorough search of the fetus for the mentioned various features to avoid misdiagnosis and establish the prognosis and the appropriate genetic counseling.

First trimester Down syndrome screening utilizing nuchal translucency measurements: Can we do it? *R.J. Wapner, L.G. Jackson, E. Pergament.* The FIRST Study Group, NICHD.

Purpose: First trimester screening for Down Syndrome may be best accomplished utilizing biochemistry (PAPP-A/hCG) and nuchal translucency (NT) measurements. NT represents an important component of this screening paradigm and may increase sensitivity to 90%. However, obtaining accurate, consistent, and standarized NT measurements may be difficult. We have evaluated the ability to standarize NT measurements at multiple sites. Methods: From May 1998, the NICHD sponsored FIRST project evaluated first trimester screening using PAPP-A, free Beta hCG, and NT. Sonographers underwent didactic education followed by performance of 50 measurements with central review. Ongoing quality evaluation was performed by review of the means and SDs of each sonographer. Sonographers deviating from the expected measurements received remedial education. Results: The table demonstrates the mean NT measurements and the percentage of measurements beneath the fifth percentile. Up to one year of hands-on measurements was required to standardize results. Conclusions: If first trimester Down Syndrome screening utilizing NT measurement is to be accomplished, standardization and continuous quality management is essential. Development of national approaches to assure this are required.

	N	N	N	Mean NT	SD	%	%
	Centers	Techs	log 10MoM			<5th%tile	>95th%tile
May-Dec 98	483	6	11	-.09	.17	17	2
Jan-May 99	783	8	20	-.08	.17	13	4
June-Aug99	724	10	27	-.05	.13	8	2

Accelerating the natural history of neurodegeneration in Huntington disease YAC transgenic mice: Facilitation of in vivo screening of potential therapeutic agents. *B.R. Leavitt¹, C.A. Gutekunst², R. Oh¹, E. Chan¹, N. Bissada¹, S. Hersch², M.R. Hayden¹.* 1) CMMT, Univ British Columbia, Vancouver, BC., Canada; 2) Emory U School of Medicine, Atlanta, GA, USA.

Effective therapy for Huntington disease (HD) will require prevention of the selective neurodegeneration underlying this disorder. Appropriate in vivo systems for screening potential therapeutic agents are required and should incorporate the disease-causing defect and use striatal neurodegeneration as an endpoint. We developed yeast artificial chromosome (YAC) transgenic mice expressing normal or mutant human huntingtin (htt) in a developmental and tissue specific manner identical to endogenous htt. YAC72 mice containing 1-2 copies of the human HD transgene with 72 CAG repeats express half the endogenous level of the mutant human protein. Selective degeneration of medium spiny neurons in the striatum, similar to that observed in HD, occurs in these mice by 12 months of age. Huntingtin aggregates are not seen by light microscopy until 18 months of age, well after the onset of neurodegeneration. The late-onset neurodegeneration in these mice limits their utility for in vivo screening of therapeutic agents. To overcome these limitations, six new lines of YAC72 mice with increased transgene copy numbers and a range of mutant htt expression from 1 to 4 times the endogenous level have been generated. Four lines of YAC transgenic mice expressing high levels of mutant human htt with 128 CAG repeats have also been generated. The YAC lines with the highest levels of mutant htt expression have massive striatal neurodegeneration beginning between 4 and 6 months of age without obvious nuclear aggregates. No neurodegeneration was observed in transgenic mice expressing equivalent levels of human htt with 18 CAG repeats. Trials of intra-striatal growth factor treatment, which previously required 9 months to complete using low-expressing YAC72 mice, can now be performed in 3 months using the high-expressing YAC72 mice. These new lines of YAC transgenic mice allow rapid and efficient in vivo screening of potential therapeutic agents utilizing striatal neurodegeneration as the primary outcome measure.

Manipulation of splicing restored dystrophin production in myocytes from a Duchenne muscular dystrophy patient. *M. Matsuo*¹, *Y. Takeshima*², *Z.A.D. Pramono*¹, *Yi. Ishikawa*³, *Ya. Ishikawa*³, *R. Minami*³, *L.P. San*⁴, *H.*

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Duchenne muscular dystrophy (DMD) is a rapid progressive skeletal muscle disease that is characterized by the complete absence of dystrophin due to out-of-frame deletion mutations in the dystrophin gene. In spite of intensive studies, no successful method of re-establishing dystrophin synthesis in DMD-derived myocytes has been established. We report here the first evidence that correction of the translation reading frame leads to production of truncated dystrophin in myocytes from DMD. In this study, a 31-mer oligodeoxynucleotide complementary to the splicing enhancer sequence of exon 19 of the dystrophin gene was transfected to cells from two DMD cases who have a deletion of exon 20. The transfection induced exon 19 skipping in a large proportion of dystrophin transcripts in Epstein-Barr Virus-transformed lymphoblastoid cells, creating an in-frame transcript lacking both exons 19 (88 nt) and 20 (242 nt). In order to demonstrate dystrophin synthesis from the in-frame transcript thereby created, myocytes were transfected with the antisense oligonucleotide. A clear signal of FITC-labeled oligonucleotide was detected in the nucleus and a proportion of dystrophin transcript lacked exon 19 as a result of the transfection. Markedly, the cytosol of more than 15 % of myocytes stained positive for dystrophin with monoclonal antibody against the C-terminal domain of the protein, while no dystrophin-positive myocytes were identified without transfection. These results point to a novel way to produce dystrophin in DMD-derived myocytes by modifying the translational reading frame using an antisense oligonucleotide.

Enzyme therapy for lysosomal acid lipase deficiency in the mouse model. *H. Du¹, J. Mishra¹, B. Jarrold¹, M. Heur¹, S. Schiavi², M. Levine², D. Witte³, G. Grabowski¹.* 1) Div. of Human Genetics, Children's Hospital Medical Ctr, Cincinnati, OH; 2) Genzyme Corporation, Boston, MA; 3) Div. of Pathology, Children's Hospital Medical Ctr, Cincinnati, OH.

Lysosomal acid lipase (LAL) is the critical enzyme for the hydrolysis of the triglycerides (TG) and cholesteryl esters (CE) in lysosomes. Its deficiency produces human two phenotypes: Wolman disease (WD) and cholesteryl ester storage disease (CESD). We created a LAL null (*lal*^{-/-}) mouse model that closely mimics the human WD/CESD. To test the potential for enzyme therapy, mannanose terminated human LAL (phLAL) was expressed in *Pichia pastoris*, purified, and administered via tail vein to *lal*^{-/-} mice. Mannose receptor (M-R) dependent uptake and lysosomal targeting of phLAL were evidenced ex-vivo using M-R positive J774E cells, a murine monocyte/macrophages, and immunofluorescence staining. Following IV injection (30 min), phLAL was detected in Kupffer cells, lung macrophages, and intestinal macrophages in *lal*^{-/-} mice. At 2 mos, *lal*^{-/-} mice began received phLAL (1.5 U/dose) every third day for 10 dose. After 30 days, the yellow hepatic color was then nearly normal. The liver weight decreased by ~50%; and was about 1.5 times normal size. Histologic analyses of variety tissues from phLAL treated and untreated mice clearly showed reductions of lipid stored Kupffer cells and macrophages of the small intestine and spleen. Biochemically, TG and cholesterol in liver, spleen and small intestine were reduced by ~50%; in liver, 69%; in spleen, and by 50%; in small intestine. These studies provide "proof of principle" for phLAL enzyme therapy in human WD and CESD.

Successful prenatal transplantation of adult mouse hepatocytes into fetal mice: A potential model for prenatal treatment of hepatic enzymopathies. *R. Cywes¹, K. Tsuchiya², M. Summar³.* 1) Surgery, Vanderbilt Univ. Med. Cnt, Nashville, TN; 2) Pathology, Vanderbilt Univ. Med. Cntr, Nashville, TN; 3) Depts of Molecular Physiology and Pediatrics, Div. of Medical Genetics, Vanderbilt Univ. Med. Cntr., Nashville, TN.

There are a wide range of disorders of hepatic enzyme function. Many of these disorders are detectable by prenatal diagnosis in families at risk, and are good candidates for intervention. Past studies have shown that the infusion of hepatocytes into the hepatic system results in engraftment in the liver. However, this procedure is complicated by immune rejection similar or worse than that of whole organ transplantation. The immune status of the fetus is different than that of the child or adult and contains a window of opportunity in which transplanted cells may be accepted. By exploiting the immune "nave" window in the fetus, when there is no immune distinction between self and non-self, allogeneic hepatocytes, which contain a normal hepatic expressed gene, may be placed in the defective fetus without the need for immunosuppression. Adult mouse hepatocytes were isolated after cannulation of the liver and injection with trypsin. The cells were centrifuge gradient separated and the macrophages discarded. The donor cells were treated with DI1 which served as a fluorescent marker for donor cells. Gravid mice (< day 18 gestation), of a different strain, were anesthetized and the unborn pups exposed. One hundred microlitres of donor hepatocyte suspension was injected into the peritoneal cavity of the fetuses who were then marked with india ink. Examination of the liver of 3 day old transplanted mice showed DI1 fluorescent patterns consistent with cells derived from the donor hepatocytes. Further experiments are underway to test the longevity, function, and degree of engraftment of these cells. This technique may serve as a model for prenatal correction of hepatic enzymopathies such as urea cycle disorders, MSUD, hemophilia and organic acidemias. It should also be possible to "retransplant" these animals with cells from the same donor organ cryogenically preserved.

Response to enzyme replacement therapy in Gaucher disease patients with N370S/N370S, N370S compound heterozygotes and L444P/L444P genotypes. *J. Charrow, H.C. Andersson, P. Kaplan, E. Kolodny, P. Mistry, G. Pastores, B. Rosenbloom, R. Scott, R. Wappner, N. Weinreb.* International Collaborative Gaucher Group Registry.

To observe possible differences in clinical response to ERT in Gaucher disease as a function of genotype, we analyzed longitudinal data from all Gaucher Registry patients with N370S/N370S (N=204), N370S compound heterozygotes (N=250), and L444P/L444P genotypes (N=46). In general, L444P/L444P patients were much younger at first infusion (mean=3.8 years, SD=4.7) N370S/N370S (mean= 39.7 years, SD=20.5) or N370S heterozygotes (mean=25.4 years, SD=16.6). At baseline (relative to ERT infusion), L444P/L444P patients had a statistically significantly lower mean hemoglobin than N370S/N370S or N370S compound heterozygotes; this difference was not observed for platelet count. N370S/N370S or N370S compound heterozygotes had statistically significantly greater liver and spleen volumes at baseline than L444P/L444P. Hemoglobin response to ERT was greater in N370S compound heterozygotes than in the other groups at 6, 12, 24, 36, 48, and 60 months after first infusion, with a mean hemoglobin change (g/dl) at 60 months of +2.46, +2.45 and +2.96 in N370S/N370S, N370S compound heterozygotes and L444P/L444P genotypes respectively. Platelet counts showed a similar pattern of increase with a mean % change (1000/mm³) of 93%, 88%, and 116% , respectively. Liver volume mean % change at 48 months was -34%, -35%, and -63%, respectively. Spleen volume mean % change at 36 months was -44%, -49%, and -72%, respectively. We conclude that hemoglobin, platelet, and liver and spleen volumes show greater responses to ERT in L444P/L444P patients than in patients with a N370S mutation. These greater ERT responses in L444P/L444P may be partially explained by two factors: the greater burden of disease in L444P/L444P patients than in N370S allele patients, and the higher mean dose at first infusion for the L444P/L444P patients (61% of whom have reported Type 3 disease). These data give clinicians a useful guide for approximate expectations of responses to ERT in patients with the N370S and L444P mutations.

Preclinical Studies of Enzyme Replacement Therapy in the Mouse Model of Types A and B Niemann-Pick Disease. *E.H. Schuchman, S.R.P. Miranda, X. He, C.M. Simonaro, R.J. Desnick.* Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY.

Types A and B Niemann-Pick disease (NPD) are lysosomal storage disorders due to the deficient activity of acid sphingomyelinase (ASM). Type A NPD is a neurodegenerative disorder of infancy, while Type B NPD is characterized by the lack of neurological involvement and a phenotypic spectrum ranging from severe monocyte/macrophage disease and early demise, to a milder condition of adulthood. Mouse models of Types A and B NPD also have been constructed by targeted disruption of the murine ASM gene (ASMKO mice). Recombinant human ASM (rhASM) was purified from the media of overexpressing Chinese hamster ovary (CHO) cells and intravenously injected into 16 five month old ASMKO mice every other day for 14 days. The sphingomyelin content and histopathological lesions were markedly reduced in the hearts, livers, and spleens of these animals by this short-term enzyme treatment. A group of 10 additional ASMKO mice were then intravenously injected with rhASM for 15 weeks, starting at 3 weeks of age. Although anti-rhASM antibodies were produced in these mice, the antibodies were not neutralizing and no adverse effects were observed. Weight gain and rota-rod performance were slightly improved in the treated animals, but significant neurological deficits were still observed and their life-span was not extended by the enzyme treatment. In contrast to these CNS findings, striking histological and biochemical improvements were found in the reticuloendothelial system of the treated animals. These studies indicate that enzyme replacement therapy should be an effective therapeutic approach for Type B NPD, but is unlikely to prevent the severe neurodegeneration associated with Type A NPD.

GDNF AND CT-1 GENE THERAPY PROLONGES THE SURVIVAL OF SOD-1 MICE. *G. Acsadi^{1,2}, R. Anguelov¹, H. Yang¹, G. Toth¹, A. Jani¹, RA. Lewis¹, ME. Shy^{1,2}.* 1) Dept Pediatrics & Neurology, Wayne State Univ, Detroit, MI; 2) Center for Molecular Medicine and Genetics, Wayne State Univ, Detroit, MI.

Amyotrophic lateral sclerosis [ALS] and spinal muscular atrophy [SMA] are the most prevalent motor neuron disorders. The primary defect is related to a degeneration of motor neurons [MN]. Sporadic ALS has been associated with an abnormality in the astrocytic glutamate transporter and ten percent of familial cases show mutations in the Zn/Cu SOD while SMA is caused by the mutations in the survival motor neuron gene. Currently, there is no effective therapy available for ALS or SMA. Glial cell-derived neurotrophic factor [GDNF] and cadiotrophin [CT-1] has been shown to be the most potent factors for preventing motor neuron degeneration after injury when delivered to the MN. In this study, we have used a recombinant adenovirus vector [AVR] expressing reporter genes [GFP] and GDNF or CT-1 to study the effect of gene transfer in SOD-1 mice (a mouse model for ALS) by intramuscular administration into newborn or adult mice. We followed the clinical course and physiology (RotaRod-test; needle EMG) of the treated and untreated mice. We show that the GDNF/CT-1 and GFP expression was present in muscles of neonatally injected mice for at least 4 month. The intramuscular GDNF or CT-1 gene transfer prolonged the survival of the AVR treated SOD-1 mice by 10-20 days compared with control SOD-1 mice. GDNF gene transfer was more effective than CT-1 in the survival of these mice. EMG data revealed that the grade of fibrillation was less severe in muscles of AVR-GDNF or CT-1 injected SOD-1 animals when compared to age matched control SOD-1 mice. The histology of spinal motor neurons and ventral roots are being evaluated in both groups. In conclusion, AVR-mediated GDNF and CT-1 gene transfer may have a potential in therapy of ALS and perhaps other motor neuron diseases like SMA. Supported by MDA.

Treatment of spinal muscular atrophy by compound S. *J. Chang*¹, *H. Lee*², *H.M. Hsieh*², *Y.J. Jong*³, *N.M. Wang*¹, *C.H. Tsai*¹. 1) Department of Medical Research, China Medical College Hospital, Taichung, Taiwan; 2) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; 3) Division of Pediatric Neurology, Department of Pediatrics and Clinical Laboratory, Kaohsiung Medical College, Kaohsiung, Taiwan.

Spinal muscular atrophy (SMA) is an autosomal recessive disease characterized by degeneration of the anterior horn cells of the spinal cord leading to muscular paralysis with muscular atrophy. No effective treatment of this disorder is available at present. Studies of correlation between disease severity and the amount of SMN protein showed inverse relationship. We report here that compound S can effectively increase the exon 7-containing SMN protein in SMA lymphoblast. The mechanism to increase exon7-containing protein is through changing the alternating splicing pattern of exon 7 of SMN2 gene. In vivo study of compound S treatment of SMA-like mice also showed increased expression of SMN protein in spinal cord tissue, and the clinical symptoms in SMA-like mice also showed significant improvement after compound S treatment. Oral administration of compound S to intercross of the heterozygous pregnant knockout-transgenic SMA-like mice, showed that the birth rate of type 1 SMA-like mice was decreased in number, and SMA symptoms were ameliorated for all the three types of SMA-like mice. From these results, we suggest that compound S could be an effective drug for the treatment of human SMA patients.

Non-toxic ubiquitous overexpression of utrophin in the *mdx* mouse. *R. Fisher¹, J.M. Tinsley^{2,3}, S.R. Phelps¹, E.R. Townsend², S.E. Squire², K.E. Davies¹.* 1) MRC Functional Genetics Unit, Human Anatomy and Genetics, South Parks Road, Oxford, U.K; 2) Human Anatomy and Genetics, South Parks Road, Oxford, U.K; 3) MRC Mammalian Genetics Unit, Harwell, Didcot, Oxfordshire, OX11 0RD, U.K.

Duchenne muscular dystrophy (DMD) is an inherited, severe muscle wasting disease caused by the loss of the cytoskeletal protein, dystrophin. Patients usually die in their late teens or early twenties of cardiac or respiratory failure and there is currently no effective therapy. Ways of delivering dystrophin using viral vectors are being developed but there are problems with the immune response to the virus as well as the delivery of dystrophin to all muscle tissues.

We have shown that the dystrophin related protein, utrophin, can prevent the muscle pathology in the *mdx* mouse model of the disease which lacks dystrophin. Utrophin is usually present at the synapse in adult muscle but could be upregulated and bind at the sarcolemma to replace dystrophin. We propose the upregulation of utrophin as a promising approach for the therapy of DMD and we are screening promoter constructs attached to reporter molecules with libraries of chemicals. One important question is whether upregulation in other tissues is toxic. To test this, we generated *mdx* mice transgenic for the utrophin gene under the control of the ubiquitin C promoter. Histopathology of 20 tissues including kidney and brain did not show evidence of toxicity at levels where marked improvement was observed in the muscle pathology.

The transgenic mice are still living healthily like the normal controls after 2 years of age while most of the *mdx* control mice died at about 23 months old showing that the transgene is still conferring its therapeutic benefits and that there are no long term toxic effects. These results suggest that a tight control over the expression of utrophin may not be necessary for the correction of muscular dystrophy. These findings have important implications for the feasibility of the upregulation of utrophin via chemical compounds for the therapy of DMD.

***In vitro* correction of glycosaminoglycan storage in human and mouse MPS IIIB cell cultures using AAV-mediated recombinant α -N-acetylglucosaminidase.** H. Fu, J. Muenzer. Dept Pediatrics, Univ North Carolina, Chapel Hill, NC.

Mucopolysaccharidoses type IIIB (MPS IIIB) is due to the deficiency of the lysosomal enzyme α -N-acetylglucosaminidase (NaGlu), which results in lysosomal accumulation of glycosaminoglycans (GAG). This project is to study the application of adeno-associated virus (AAV) vectors for gene therapy in MPS IIIB. Two recombinant AAV (rAAV) vectors were constructed, containing a human NaGlu coding region cDNA, driven by a human cytomegalovirus (CMV) immediate early promoter or a neuron-specific enolase (NSE) promoter. The rAAV-CMV-hNaGlu was shown to be able to mediate *in vitro* expression of functional secretory NaGlu in human MPS IIIB fibroblasts, and mouse MPS IIIB brain, liver, skin fibroblast and kidney primary cell cultures. *In vitro* correction of GAG storage was studied using $^{35}\text{SO}_4$ -labeling. Complete correction of GAG storage was observed in human MPS IIIB fibroblasts, when transduced with rAAV-CMV-hNaGlu, or incubated with 2 units of NaGlu activity/ml of rNaGlu in the media. Partial correction (80-95%) of GAG storage was seen in MPS IIIB mouse brain, liver, skin fibroblast and kidney primary cell cultures, when infected with rAAV-CMV-hNaGlu. However, only limited correction (30-40%) of GAG storage was observed when 5 units of NaGlu activity/ml of rNaGlu were added to the media of mouse cell cultures. rAAV-NSE-hNaGlu mediated enzyme expression in mouse MPS IIIB primary brain cell cultures was not as efficient as rAAV-CMV-hNaGlu. The decreased expression using the neuron-specific promoter may be because the majority of cells in primary brain cell cultures are glial cells. In summary, the rAAV-CMV-hNaGlu vector can mediate efficient expression of rNaGlu in both human MPS IIIB fibroblasts and mouse MPS IIIB primary cell cultures. However, the human rNaGlu secreted by AAV-transduced human or mouse MPS IIIB cells is more efficiently taken up by human MPS IIIB cells than by mouse MPS IIIB cells. (This project is supported by a grant from The Children's Medical Research Foundation, Inc., Western Springs, IL).

Fragile X transgene and embryonic lethality in mice. *W.T. Brown¹, X. Ding¹, A.E. Idrissi¹, J. Scalia¹, A. Glicksman¹, R. Bauchwitz², C. Dobkin¹.* 1) Dept Human Genetics, NYS Inst Basic Research, Staten Island, NY; 2) Department of Genetics and Development, Columbia University, NY, NY 10038.

An understanding of the potential adverse effects of fragile X gene overexpression is important for the rational design of gene therapy approaches to this disorder. Transgenic mice carrying a 100 kb fragment including the entire human fragile X gene, FMR1, and flanking DNA were engineered to study the effects of additional expression of this gene. A founder with 1-2 copies of the gene was analyzed. FMR1 mRNA transcription was found to be approximately equivalent to that of the endogenous mouse Fmr1 gene in both quantity and tissue specificity. Repeated backcrossing of this transgenic line onto an FVB/N background yielded fewer than expected transgenic females. Instead of the expected 50%, only 28% (20 of 71) of the female offspring of heterozygous transgenic mothers carried the transgene ($Z = -3.68$, $p < 0.0002$). A small reduction in the average litter size implies that embryonic lethality leads to a loss of approximately 1 transgenic female per litter. The frequency of male transgenic mice (52%) did not appear to be affected. These results are apparently consistent with observations by others of reduced FMR1 transgene transmission in an independently derived C57BL/6 line [Peier et al. 2000, Hum. Mol. Genet. 9, 1145-1159]. Analysis of the developmental stage at which lethality occurs and morphological abnormalities are being determined to identify the developmental effects of fragile X gene overexpression. Transmission on an Fmr1 knockout mouse background is being studied to see if reduced expression of the endogenous Fmr1 gene influences the frequency of transgenic females. The embryonic lethality implied by these results suggests that control of expression level will be important for fragile X gene therapy approaches.

Functional recovery and differential cellular immune response following AAV gene delivery to a- and b-sarcoglycan deficient muscle. *D. Dressman*^{1,2}, *T. O'Day*³, *J. Watchko*³, *L. Liu*⁴, *E. Engvall*⁴, *K. Araishi*⁵, *M. Imamura*⁵, *T. Sasaoka*⁵, *E. Ozawa*⁵, *E.P. Hoffman*^{1,2}. 1) Ctr for Genetic Med, CNMC, Washington DC; 2) U Pittsburgh, Molecular Genetics & Biochem, PA; 3) Magee-Women's Res Inst, Pitt, PA; 4) Burnham Inst La Jolla CA; 5) National Inst Neuroscience, Tokyo Japan.

Adeno-associated virus (AAV) shows more promise than any other vector for gene delivery to mature skeletal muscle. We have recently shown complete functional recovery of the tibialis anterior (TA) muscle of d-sarcoglycan deficient hamsters, after AAV gene delivery (Xiao et al. 2000). However, d-sarcoglycanopathies are the most rare muscular dystrophy characterized to date, with only three unrelated patients reported. We have shown that of the sarcoglycanopathies, a-sarcoglycanopathies occur the most frequently, followed by b-sarcoglycanopathies (Duggan et al. 1997). Here, we report the first construction of recombinant AAV for a- and b-sarcoglycan gene delivery. Human full-length cDNAs for a- and b-sarcoglycan were cloned into an AAV vector under a CMV promoter from muscle biopsies and sequence verified. AAV was produced by an adeno-free, triple transfection method. Mouse K/O's for both a- and b-sarcoglycan were obtained, and single injections were made into the TA muscles of adult mice. Biochemical, histological and functional studies were done 4 wks after injections. All seven AAV-treated a-sarcoglycan-deficient TA muscles showed significant expression of a-sarcoglycan in the complete TA muscle (10-40% of fibers). However, substantial inflammation was present in all muscles examined, suggesting that expression of human a-sarcoglycan induces an aggressive cellular immune response; this is in contrast to most other reports of gene delivery by AAV. Despite the inflammatory response, functional testing showed injected muscles (n=7) to be statistically significantly stronger than controls (n=16). For b-sarcoglycan deficiency, the three muscles studied showed high level expression in 5-30% of fibers, with no evidence of inflammation. These results are the first to demonstrate biochemical and functional rescue of muscular dystrophy types which are at a relatively high incidence in non-consanguineous human populations.

Engraftment of genetic-engineered amniotic epithelial cells corrects lysosomal storage in the whole brains of mice with mucopolysaccharidosis type VII. *M. Kosuga*^{1, 2}, *X-K. Li*³, *H. Sago*⁴, *M. Kitagawa*⁴, *S. Suzuki*³, *M. Yamada*¹, *N. Matsuo*², *T. Okuyama*^{1, 2}. 1) Genetics, Natl. Children's Med. Res. Cen, Tokyo, Japan; 2) Dept. Ped., Keio Univ., Tokyo, Japan; 3) Dept. Exp. Surgery, Natl. Children's Med. Res. Center; 4) Dept. OB&GYN, Natl. Okura Hosp., Tokyo, Japan.

Cell-mediated therapy for visceral lesions of lysosomal storage diseases is promising, however, treatment of the CNS lesions is still challenging. In this study, we first showed that amniotic epithelial (AE) cells infected with E1-deleted adenoviral vectors survived for more than six months at the transplanted corpus striatum of the mice brains. This long survival tendency was utilized for the cell-mediated therapy for CNS lesions of the mice with mucopolysaccharidosis VII (MPSVII), a lysosomal storage disorder by an inherited deficiency of b-glucuronidase (GUSB). The cells were transduced with an adenoviral vector expressing human GUSB, and generated AE cells over-expressing and secreting the enzyme. After confirming that the GUSB from the AE cells was taken up mainly via mannose 6-phosphate receptors in primary cultured neurons, the cells were transplanted into adult MPSVII mice brains. Histochemical study showed extensive distribution of GUSB-positive cells throughout the ipsilateral hemisphere of the recipient brain, and pathological improvement of the lysosomal storage was observed even at the region far away from the site of injection. These results indicate that intra-cerebral transplantation of genetic-engineered AE cells have therapeutic potential for the treatment of CNS lesions in lysosomal storage disorders.

Preclinical Studies of Hematopoietic Stem Cell Gene Therapy in the Mouse Model of Types A and B Niemann-Pick Disease. *S.R.P. Miranda, E.H. Schuchman.* Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY.

Types A and B Niemann-Pick Disease (NPD) are lysosomal disorders due to the deficient activity of acid sphingomyelinase (ASM). Type A NPD is a severe neurodegenerative disorder which generally leads to death by three years of age. In contrast, Type B NPD is characterized by the lack of neurological involvement, variable peripheral organ system disease, and survival into adolescence or adulthood. Currently, no treatment is available for either form of NPD. We evaluated the efficacy of hematopoietic stem cell (HSC) mediated gene therapy for these disorders using the mouse knockout model of Types A and B NPD (ASMKO mice). Thirty-two newborn ASMKO mice were preconditioned with low dose radiation (200 cGy) and transplanted with ASMKO bone marrow cells which had been transduced with an ecotropic retroviral vector encoding human ASM. Engraftment of donor-derived cells ranged from 15-60% based on Y-chromosome in situ hybridization analysis of peripheral white blood cells, and was achieved in 92% of the transplanted animals. High levels of ASM activity were found in the engrafted animals for up to 10 months post-transplant, and their life-span was extended from a mean of ~5 to 9 months. Biochemical and histological analysis obtained 4-5 months post-transplant indicated that the ASM activities were increased and the sphingomyelin storage was significantly reduced in the spleens, livers and lungs. The presence of Purkinje cell neurons also was increased in the treatment group at 5 months post-transplant, and a reduction in spinal cord storage was evident. However, all of the transplanted mice eventually developed ataxia and died earlier than normal mice. Overall, these results indicated that HSC gene therapy should be effective for the treatment of non-neurological Type B NPD, but improved techniques for targeting the transplanted cells and/or expressed enzyme to specific sites of pathology in the central nervous system must be developed in order to achieve effective treatment for Type A NPD.

Correction of factor VIII deficiency in hemophilia A mice that target expression of a single chain variant of human factor VIII to epidermis. *S. Kobayashi*¹, *R. Sarkar*², *H.H. Kazazian, Jr.*², *S.S. Fakharzadeh*¹. 1) Department of Dermatology and; 2) Department of Genetics, University of Pennsylvania, Philadelphia, PA.

Hemophilia A is an X-linked bleeding disorder caused by lack of functional factor VIII protein, making it a good model disease for gene therapy. Epidermis is an attractive target tissue for gene therapy because it is accessible and gene products expressed there can enter the systemic circulation. Previously, we generated transgenic mice that express human B domain-deleted factor VIII in the suprabasal epidermis under control of the involucrin promoter and bred them into a factor VIII knockout background. These mice had functional circulating factor VIII and displayed phenotypic correction. This demonstrated proof of principle that targeting factor VIII expression to epidermis can correct hemophilia A. We are now exploring strategies for optimizing factor VIII expression in the epidermis to maximize delivery to and activity in the circulation. One approach is to express factor VIII as a single peptide rather than the normal precursor that is cleaved into two chains, which form an unstable heterodimer. We generated a transgene construct that uses the involucrin promoter to express a variant of human B domain-deleted factor VIII that lacks the proteolytic cleavage site at residue 1648. Eight transgenic founder mice were obtained that express this construct as shown by RT-PCR of RNA from tail tissue. To date, one line has been bred into a factor VIII knockout background. Three transgenic mice from this line showed factor VIII activity of 25-30% of normal and survived tail clipping, whereas two non-transgenic littermates showed no activity and did not survive tail clipping. Therefore, these transgenic mice produce functional factor VIII and are phenotypically corrected. We are comparing factor VIII expression, activity and antigen levels of these mice to those of mice that express standard B domain-deleted factor VIII under control of the involucrin promoter. In this way, we will assess whether epidermal expression of single peptide factor VIII is superior to two-peptide factor VIII for gene therapy.

Prevention of muscular dystrophy in mdx mice by four-repeat micro-dystrophins. *S.Q. Harper*¹, *R.W. Crawford*², *C. DelloRusso*³, *R.B. Maniker*², *J.S. Chamberlain*^{1,2}. 1) Program in Cellular and Molecular Biology; 2) Dept. of Human Genetics; 3) Dept. of Physiology, University of Michigan, Ann Arbor, MI.

Duchenne Muscular Dystrophy (DMD) is an inherited, X-linked recessive disorder characterized by progressive muscle degeneration and weakness. Death often results at an early age from respiratory failure or cardiomyopathy. The disease is caused by mutation in the dystrophin gene, which encodes a 427 kDa protein that is localized to the subsarcolemmal membrane in complex with several other cytoskeletal, integral membrane, and extracellular matrix proteins. This complex of proteins is termed the dystrophin-associated protein (DAP) complex and is thought to stabilize the muscle membrane by linking the actin cytoskeleton to the extracellular matrix. In so doing, it is believed that the DAP complex helps prevent contraction-induced injury to the muscles. One of the obstacles of current gene therapy strategies to DMD is the difficulty of cloning full-length 14 kb dystrophin cDNAs plus regulatory elements into current viral vectors. We have constructed three micro-dystrophin genes that encode only four of the twenty-four spectrin-like repeats in the central rod domain. These 4 kb micro-dystrophins were used to generate transgenic *mdx* mice to test their ability to reverse the dystrophic phenotype. The level of correction in mice expressing these transgenes was examined by histology, immunofluorescence, and functional assays. These four-repeat dystrophins are expressed at the sarcolemma and significantly reduce the amount of dystrophy in the *mdx* mouse. Additionally, there is variable correction observed among the different micro-dystrophins. For example, one mouse line expressing a micro-dystrophin transgene showed wild-type levels of central nuclei, which are an indicator of the degree of muscle regeneration. Another line showed approximately 80% correction of the dystrophy with this assay. Our ultimate goal is to identify the smallest dystrophin construct that has therapeutic potential and can be expressed in an adeno-associated viral (AAV) vector. We are currently in the process of testing these micro-dystrophin clones with recombinant AAV vectors.

Retroviral marking of canine bone marrow: high level expression of human IL-2 receptor common gamma chain and multiple drug resistance 1 in canine peripheral blood and bone marrow after Taxol therapy. *M.E. Haskins¹, J.R. Melniczek¹, T. Licht², T. Whitwam², D.M. Bodine², J.M. Puck², P.S. Henthorn¹*. 1) Section of Medical Genetics, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; 2) Genetics and Molecular Biology Branch, National Human Genome Research Institute, National Institutes of Health Bethesda, MD.

Optimizing retroviral gene transfer into hematopoietic cells of the dog will facilitate gene therapy for several canine genetic diseases and advance the treatment of humans with similar diseases. Canine and human X-linked severe combined immunodeficiency (XSCID) are caused by defects in the common γ chain (γc) of receptors for interleukin-2 and other cytokines. In this study, a normal two-month-old female dog was given retrovirally transduced bone marrow cells after pre-harvest mobilization by recombinant canine stem cell factor and recombinant canine granulocyte colony-stimulating factor. A Harvey sarcoma virus construct was used containing cDNA encoding for human γc and multiple drug resistance Type 1 (MDR1). The Harvey-based vector that was transduced into cytokine-primed marrow cells yielded persistent detectable provirus in bone marrow and blood, and expression of human γc on peripheral lymphocytes. Human γc expression disappeared after week 34, but reappeared week 42 post-transplantation after immunosuppression. Two years after the dog received the retrovirally transduced bone marrow cells, and after expression was no longer detectable, the dog was given Taxol. Three doses at 125 mg/m² IV were given over a three-month period. After the final dose of Taxol, expression of both human γc and MDR1 was seen on the surface of bone marrow cells, as well as granulocytes, monocytes, and lymphocytes in the peripheral circulation. No signs of myeloproliferative disease were observed. The re-emergence of human γc and MDR1 expression after Taxol and prior extinction suggests that undetected populations of cells expressing genes of interest can be selected and expanded in the bone marrow and peripheral blood.

Molecular therapy, another approach to gene therapy. *D.F. Schorderet¹, A. Oberson¹, S. Negri¹, C. Sauser¹, S. Stern², L. Maggiorella³, V. Frasca³, C. Bonny¹.* 1) Medical Genetics; 2) Radio-oncology, CHUV, Lausanne, Switzerland; 3) Inst. Gustave-Roussy, Villejuif, France.

Although gene therapy holds great future, each step of the cell cycle is a potential target for treatment or modulation. In order to evaluate such alternative therapies, we investigated whether molecular therapy could alter the genetic fate of a cell system. As model, we used c-Jun N-terminal kinases (JNK)-mediated apoptosis induced by interleukin-1 (IL-1) in bTC3. It was shown that JNK pathway is preferentially activated by several classes of cell surface receptors and in response to environmental stress. Blocking this pathway prevents or decreases major changes in cell fates, including oncogenic transformation, VEGF-induced endothelial cell proliferation, apoptosis of ischemic neurons and cardiomyocytes, and IL-induced apoptosis. IB1 is a scaffold protein that mediates activation of JNK through the interaction of a 280-aa JBD domain. In order to use this domain as a potential therapeutic target, we characterized the smallest active domain of JBD. The resulting 20-aa peptide was synthesized and covalently linked to the HIV-TAT sequence (Schwarze et al. 1999) to form TAT-IB1. We first evaluated whether TAT-IB1 was able to enter different cell types. Exposure of several cell lines to labeled TAT-IB1 for periods as short as 5' was followed by an important uptake of the peptide by all the cellular compartments. TAT-IB1 was then applied to IL-1 stimulated bTC3 cells and apoptosis was measured. TAT-IB1 is able to confer full protection against apoptosis. In a second experiment, the radiation protective action of TAT-IB1 was evaluated in an animal model. The lower lip of 6 TAT-IB1 treated mice was irradiated with a dose of 16.5 Gy and the loss of weight was compared to a cohort of non TAT-IB1 treated mice. Significant reduction of the weight loss was observed in the treated animals.

Our example establishes that cell-permeable peptides represent potent pharmacological compounds that can be used to modulate gene action. This approach is potentially applicable to all protein interactions and represents an alternative approach to gene therapy.

Long-term morphological normalization in whole brains of mice with mucopolysaccharidosis VII by neonatal systemic administration of an adenoviral vector expressing b-glucuronidase. A. Tanabe¹, M. Kosuga^{1, 2}, X-K. Li³, H. Sago⁴, M. Kitagawa⁴, S. Suzuki³, M. Yamada¹, T. Okuyama^{1, 2}. 1) Genetics, Natl. Children's Med. Res. Cen, Tokyo, Japan; 2) Dept. Ped., Keio Univ., Tokyo, Japan; 3) Dept. Exp. Surgery, Natl. Children's Med. Res. Center, Tokyo, Japan; 4) Dept. OB&GYN, Natl. Okura Hosp., Tokyo, Japan.

Systemic intravenous administration of an adenoviral vector into adult mice with mucopolysaccharidosis VII (B6/MPSVII) resulted in rapid amelioration of lysosomal storage in multiple visceral organs (Kosuga, Okuyama et al. Molecular Therapy 2000). However, no obvious therapeutic efficacy was obtained in the brains of the adult mice by this treatment. Since progressive mental retardation is a frequent observed symptom in MPSVII or other lysosomal storage disorders, developing the strategy to treat the CNS lesions were very important clinically. In this study, we administered an adenovirus expressing human b-glucuronidase (AxCaHUS) into newborn B6/MPSVII, and analyzed whether morphological normalization of the brain was obtained. A hundred µl solution containing 1x10⁸pfu of AxCaHUS was injected into neonatal B6/MPSVII via superficial temporal veins within 48 hours after delivery. The mice were sacrificed periodically, and GUSB expression and pathological correction was analyzed. Significant GUSB activities (80 % of their normal littermates) were detected in the brains of the treated mice even at 60 days after the treatment. Histochemical analysis showed disseminated strong GUSB-positive cells and diffuse weak positive areas were observed, suggesting that both the vector-transduced cells and cross-corrected cells co-existed in the brains. Furthermore, histopathological analysis demonstrated that complete morphological normalization could be achieved in all examined areas of the brains. Starting adenovirus-mediated gene therapy at neonatal period is favorable to prevent the development of CNS lesions in MPSVII or in other lysosomal storage disorders.

ABO/Rh(D) status, karyotype, and FISH with human-specific X and Y probes: 3 relatively simple ways for detecting chimerism after in- utero transfusion of human progenitor cells in the baboon model. *J. Santolaya¹, T. Hewitt¹, S.D. Sosler¹, P. DeChristopher¹, Z. Shen², M. McCorquodale², D.J. McCorquodale².* 1) Obstetrics & Gynecology, Univ. of Illinois at Chicago, Chicago, IL; 2) Michael Reese Hospital and Medical Center, Chicago, IL.

Due to its size, type of placentation, and the similarities of the embryonic development with humans, the baboon model may be one of the best ways for testing the feasibility of in-utero gene therapy using hematopoietic progenitor and stem cells obtained from umbilical cord blood. We propose 3 relatively simple ways of determining the presence of human cells in a baboon's newborn transfused in-utero. 1) We have tested 30 baboons for human ABO and Rh(D) antigens and all are group O, Rh(D) negative, suggesting that if A, B, or Rh(D) positive donor cells are used, simple agglutination techniques could accurately detect a degree of mosaicism greater than 5% (based on observing mixed-field agglutination) in peripheral blood, and genotyping using specific primers and PCR could detect the presence of human DNA at very low concentrations and in different tissues; 2) baboons have 42 chromosomes compared to 46 in humans (8 animals tested) which could confirm the agglutination technique; 3) fluorescence in situ hybridization using X and Y probes that are specific to human DNA and do not hybridize to baboon DNA, either in interphase or metaphase (200 baboon cells tested from each of 3 animals of different sexes using human controls). It is therefore realistic to expect that FISH analysis of 200 cells would allow for detection of 0.5% or greater incidence of tissue chimerism.

The application of antisense hammerhead ribozymes to the down-regulation of the COL1A1 gene in an animal model of osteogenesis imperfecta. *I. Toudjarska*¹, *M.W. Kilpatrick*¹, *J. Niu*¹, *R.J. Wenstrup*², *P. Tsipouras*¹. 1) Pediatrics, UConn Health Center, Farmington, CT; 2) Human Genetics, Children's Hospital Research Foundation, Cincinnati, OH.

Antisense hammerhead ribozymes are small catalytic RNA molecules that can be designed to cleave a target RNA molecule that contains a putative NUY cleavage site (where N is any nucleotide and Y is any nucleotide except G). This ability has led to the application of antisense hammerhead ribozymes to the down-regulation of a variety of gene products, and to the proposal that such ribozymes might be applied to the selective elimination of mutant gene products particularly in situations where the mutant product exerts a dominant-negative effect. Osteogenesis Imperfecta is, in almost all cases, caused by a mutation in one of the genes for type 1 collagen (COL1A1 and COL1A2) and there is considerable evidence that the more severe forms of the disease are caused by the mutant collagen molecule exerting a dominant-negative effect. Hammerhead ribozymes targeted to mutant type 1 collagen are therefore potential therapeutic agents for OI. Hammerhead ribozymes were designed to specifically target a COL1A1 minigene which is deleted for exons 6 through 46. Expression of this minigene in the murine calvarial cell line MC3T3-E1 and in transgenic mice results in reproducible biochemical and phenotypic abnormalities. The ribozyme COL1A1RZ547, designed to cleave at the junction of COL1A1 exons 5 and 47 which is present only in the minigene, cleaved its target with high efficiency. The ability of the ribozyme to selectively cleave its minigene target in total RNA isolated from MC3T3-E1 cells was quantitated by RNase protection. The ribozyme was highly specific for its mutant collagen target and did not cleave normal collagen transcript. The ribozyme was cloned into an expression vector which co-expressed gfp allowing the delivery of the ribozyme to MC3T3-E1 cells to be monitored, along with the effect of the ribozyme on its minigene target. This construct is being used to stably transfect MC3T3-E1 cell lines so the ability of the ribozyme to reverse the mutant cellular phenotype can be determined.

Helper-dependent adenoviral vectors for gene therapy of atherosclerosis. *L. Pastore¹, K. Oka², M. Belalcazar², I.-H. Kim², A. Merched², R. Cela¹, B. Lee¹, A.L. Beaudet¹, L. Chan².* 1) Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept. of Molecular and Cellular Biology, Baylor College of Medicine.

In previous work using helper-dependent adenoviral (HD-Ad) vectors and human alpha1-antitrypsin as a reporter gene, we have demonstrated very long-term expression in mice and baboons and supraphysiological levels in mice with no evidence of toxicity (Hum Gene Ther, 9:2709, 1998; Proc Natl Acad Sci USA, 96:12816, 1999). Our goal is to use HD-Ad vectors to achieve very long-term and high level expression to prevent and/or reverse atherosclerosis particularly with the goal of expressing the LDL receptor and apolipoprotein A-I (ApoA-I) in human subjects. In preparation for clinical trials, experiments were performed in mice using HD-Ad vectors expressing the cDNA for mouse VLDL receptor with a PEPCK promoter (HD-Ad-mVLDLR) or human genomic ApoE (HD-Ad-hApoE) or human genomic Apo A-I (Hd-Ad-hApoA-I). Expression of mVLDLR in mice lacking LDL receptor (LDLR) resulted in lowering of cholesterol for greater than six months and nearly complete prevention of atherosclerotic lesions with no evidence of toxicity. Expression of Apo A-I in mice lacking Apo A-I reversed the lipoprotein phenotype, resulting in correction for over 20 months, and expression in mice lacking LDLR reduced atherosclerotic lesions substantially. Supraphysiological levels of Apo A-I (up to 200 mg/dl) were achieved and expression lasted more than six months, resulting in increase in HDL cholesterol in mice lacking ApoE or LDLR. Expression of ApoE in mice lacking ApoE resulted in correction of the lipoprotein phenotype. In conclusion, overexpression of anti-atherogenic proteins with HD-Ad vectors is a safe and effective method to improve lipoprotein profile and reduce lesions in atherosclerosis-prone mice.

Relationship of splice site information content to severity of hemophilia A and B. *Y.v. Kodolitsch¹, I. Schalwat¹, S.R. Sjojanovsky², E. Berger¹, P.K. Rogan².* 1) Dept Cardiol, Univ Hosp Eppendorf, 20246 Hamburg, Germany; 2) Sect Mol Genet & Mol Med, Children's Mercy Hosp, Univ Missouri-Kansas City Sch Med 64108.

Protein reduction and bleeding symptoms vary widely among different mutations in the factor VIII (*F8C*) or factor IX (*F9*) genes. Predicting disease severity by mutation analysis may be useful in titrating *F8C* and *F9* levels in replacement therapy. Individual information-based models (R_i) of splicing have been used to predict severity of *F8C* and *F9* splicing mutations, many of which are located within splice sites or create novel splice junctions (71 of 584). Mutant sites with R_i values <2.4 bits or with >100 fold reduction in strength ($DR_i > 6.6$) are inactivated and usually produce severe phenotypes, whereas leaky mutations with decreased splicing have R_i values >2.4 bits, <100 fold decrease in splice site strength, and produce milder symptoms. Of 61 distinct splice variants ($n=71$), loss of splice site recognition was predicted for 43, decreased splicing for 15, and unaltered R_i values for 3 variants. Cryptic splicing was predicted for 12 of these mutations. The predictions were compared with measures of protein activity and clinical severity. Factor VIII and IX protein activities $<1\%$ of normal were graded severe, moderate was between 1 and 5%, and mild was $>5\%$. R_i analysis accurately predicted reduction in protein activity for 50 mutations (70%). Reduction in activity was overestimated for 13 mutations predicted as severe, and was underestimated for 5 mutations predicted to be leaky. There was concordance (18/18) between mutations predicted to be severe and clinical severity based on frequency of bleeding symptoms, but only 5 of 8 leaky mutations were clinically mild, an overall accuracy of 88%. For cryptic splicing mutations, differences between the strengths of cryptic and mutant natural sites diminished residual splicing at the natural site. When these differences were considered, the predictions corresponded to the observed reduction in protein activity for 15 of 18 mutations (10/18 if the cryptic site was not considered). In summary, R_i analyses correctly discriminated 74% of severely affected patients carrying splicing mutations from those with milder disease.

Treatment of type 3 Gaucher disease using bone marrow transplantation. *J.J. MacKenzie¹, C.R. Greenberg², J.T.R. Clarke³.* 1) Dept of Pediatrics, Queen's University, Kingston, ON; 2) Depts of Pediatrics and Child Health & Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, MN; 3) Dept of Pediatrics, University of Toronto, Toronto, ON.

Gaucher disease, due to a deficiency of the enzyme glucocerebrosidase, is the most common lysosomal storage disorder. Type 3, the subacute neuronopathic form, generally presents with reticuloendothelial storage followed by the onset of neurological symptoms. Neurologic involvement classically develops in later childhood with a horizontal gaze apraxia as the initial feature. Effective enzyme replacement therapy (ERT) has saved the lives of many children with type 3b disease who formerly would have died from the systemic involvement before developing significant neurologic impairment. We report here two cases of children who presented with severe systemic Gaucher disease at 2 years of age who later developed classical signs of neurologic involvement. Genotypes were L444R(1448G)/unknown and G377S(1246A)/E326K(1093A). ERT was initiated in both cases shortly after diagnosis with doses ranging from 15-120 units/kg/month. Both developed oculomotor apraxia at 7 years of age while on ERT. Patient 1 had no other clinical neurological signs but mild white matter changes were noted on MRI. Patient 2 went on to develop tremor and seizures. MRI revealed bilateral parieto-occipital cystic encephalomalacia. Because of inconsistent literature regarding benefits of high dose ERT compared with bone marrow transplantation (BMT), both patients underwent BMT. Neither patient has had progression of disease (15 months and 9 months post transplant) and patient 2 has been seizure free since 3 months after the procedure. Our results suggest that BMT is a reasonable treatment option particularly if the disease progresses while the patient is on high-dose ERT.

Conditional expression of the human acid α -glucosidase (GAA) gene in GAA knockout mice. *N. Raben¹, N. Lu¹, K. Nagaraju¹, B. Byrne², P. Plotz¹.* 1) NIAMS, ARB, NIH, Bethesda, MD; 2) Gilis Institution, University of Florida.

Profound deficiency of acid α -glucosidase, known as Pompe disease, results in generalized deposition of glycogen in lysosomes and manifests as cardiomegaly and severe muscular weakness fatal in infancy. GAA knockout mice (-/-) develop pathological and clinical features similar to those in humans (Raben et al. 1998; 1999). We have now generated transgenic mice expressing human GAA on a knockout background. Two transgenic strains were generated: GAA-/- carrying a tet responsive element followed by hGAA cDNA and an IRES-EGFP sequence; GAA-/- carrying the tetracycline regulated transactivator (tTA) under the control of the MCK promoter to allow for restricted conditional expression in muscle. When the two strains are crossed, the resulting double transgenic progeny express human GAA. GFP was used to assess the expression pattern and effectiveness of the system. This expression can be abolished upon administration of doxycycline. In the four founder lines, increased levels of GAA activity in skeletal muscle were detected, ranging from 10-200 fold compared to the GAA+/- controls. Skeletal muscle offers an accessible tissue for the gene expression in vivo, and we looked for metabolic correction in distant organs in the deficient mice. In the low expressor line, there was no increase in GAA activity in the liver, kidney, lung, or brain. However, in the high expressor lines, 2-3 fold increased levels of GAA were detected in the liver, kidney, lung, and spleen. Since recent gene replacement studies in GAA-/- mice demonstrated potential benefits of hepatic transduction of the hGAA gene (Amalfitano et al. 1999), we have also generated a strain carrying tTA under the control of an albumin promoter to allow for restricted conditional expression in liver. The tetracycline-responsive gene system allows for the expression of a transgene to be turned on at different stages of the disease progression. By turning the gene on after the onset of either muscle pathology or clinical symptoms, we will determine at what stage, if at all, the disease is reversible. Finding the answer to this question can have profound implications for both enzyme and gene replacement therapies.

Gene Therapy for duchenne muscular dystrophy by hepatocyte growth factor (HGF) gene transfer. H.

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Hepatocyte growth factor (HGF) is the only known growth factor that activates quiescent satellite cells in primary culture and muscle tissue. In addition, HGF was shown to be present in muscle from mdx, a model of Duchenne muscular dystrophy (DMD), and colocalized with c-met in regions of muscle repair. We hypothesized that HGF may activate skeletal muscle satellite cells of DMD patients and result in keeping active muscle regeneration and preventing fibrosis. According to our preliminary study, mdx showed higher levels of HGF in quadriceps, diaphragm and plasma compared with control C57BL/10 between 4-12 weeks of age and highest value around 6 weeks of age. In mdx, the percentage of necrotic area of diaphragm, most severely affected muscle, was highest at 6 weeks of age (39%). These findings suggested that active muscle regeneration in mdx might be induced by HGF. To investigate the effect of HGF, activating muscle regeneration and preventing fibrosis, 50g of plasmid human HGF (pUC-SR/HGF) or the same volume of saline was injected into tibialis anterior (TA) of 3 week- or 4 week- old mdx. One week after each injection, human HGF was detected in the local plasmid HGF-injected TA. Necrotic areas of diaphragm significantly decreased in HGF-injected mdx compared with saline-injected mdx (11.3% and 3.6% at 4 weeks of age, 45.0% and 5.2% at 5 weeks of age, respectively). Immunohistochemical analyses also showed a decrease of fibrosis and an increase of muscle satellite cells. We conclude that HGF gene therapy will be a new therapy for DMD patients because HGF-injected mdx showed prevention of muscle necrosis and fibrosis and activation of muscle regeneration.

Beta-blockers may improve sleep disturbance in Smith-Magenis syndrome. *H. De Leersnyder¹, M.C de Blois¹, S. Lyonnet¹, D. Sidi², D. Genevieve¹, A. Munnich¹.* 1) Dept of Genetics, INSERM U393, Hospital Necker-Enfants Malades, Paris, France; 2) Dept of Cardiology, Hospital Necker-Enfants Malades, Paris, France.

Smith-Magenis syndrome (SMS) is a contiguous gene deletion syndrome on chromosome 17p11.2, responsible for a behavioral phenotype including mental retardation, typical facial appearance, hyperactivity, attention deficit and severe sleep disturbance. We and other have previously reported on the specific sleep phenotype in SMS. Patients have a sleep phase advance (early sleep onset, frequent awakenings, early sleep offset). They have naps at midday and sleep attacks at the end of the day. Most interestingly, they display a phase shift in their circadian rhythm of melatonin. Indeed, sleep disturbance and behavioral problems correlate with the inverted circadian rhythm of melatonin. The secretion of melatonin, the main pineal gland hormone, undergoes a circadian rhythm, entrained by light and dark variation and controlled by the suprachiasmatic nucleus of the hypothalamus and the sympathetic nervous system. Considering that i) the inverted cycle of melatonin may induce hyperactive day behavior and prevent quiet sleep in the night and that ii) beta-blockers decrease melatonin release via specific inhibition of adrenergic beta1-receptors, we have tried to block melatonin release with acebutolol, a selective beta1-blocker, given to SMS children as a single dose early in the morning, after informed consent of the parents and approval of our bioethical committee. The study included questionnaires, sleep diary, actimetry prior and after treatment. Cardiac examination, ECG, cardiac echography, 48 hrs holter recording were conducted before drug administration. Plasma melatonin levels were monitored every 2 hours before and after beta-blockers administration. Psychometric evaluation was conducted before and after 6 months treatment. Preliminary results are particularly encouraging. This pharmacological approach will hopefully help managing hyperactivity and sleep disorders in SMS children and understanding the mechanism underlying the inversion of the circadian rhythm of melatonin in this disease.

Post-gene translational research: from mouse drug trials to an international human clinical trial network in Duchenne dystrophy. *D. Escolar*¹, *E. Henricson*¹, *J. Granchelli*², *G. Buyse*³, *R. Leshner*⁴, *E.P. Hoffman*¹. 1) Children's Nat. Med. Ctr., Washington, DC; 2) SUNY Buffalo, Buffalo, NY; 3) U.Z. Gasthuisburg, Leuven, Belgium; 4) Medical College of Virginia, Richmond, VA.

We present a model for drug screening in knockout mouse models of human disease, where positive hits are studied by an international clinical trial group with standardized subject assessment and web-based data entry. Duchenne muscular dystrophy (DMD) is the most common lethal childhood genetic disorder, affecting 1/3500 male newborns. Despite a good molecular understanding of the disease since 1986 no increase in survival has occurred for affected children. We developed an international bench to bedside translational research group that uses laboratory discoveries to develop targeted pharmacological interventions, reviews them for efficacy and safety in animal models, and moves promising interventions directly to early-stage clinical trials or further animal model toxicity studies. Potential interventions are tested in the dystrophin-deficient mouse model of DMD at the SUNY Buffalo laboratory of Dr. Granchelli. Mice are exercised by involuntary treadmill running (30 min. twice per week), whereupon measurable weakness is expressed. Among 56 drugs given IP daily for 3 months, significantly increased strength is seen with creatine, glutamine, oxatomide, IGF-1, pentoxifylline, CoQ 10, pyridoxine, taurine and IL-1ra. Hit drugs are developed into human subject safety and efficacy studies by the Cooperative International Neuromuscular Research Group (CINRG), or undergo further toxicity studies in animal models. We show a web-based system for strength assessment and show dramatic increase in reliability of these methods as compared to traditional manual muscle testing in a 12-center clinical evaluator reliability study. We describe a central infrastructure for clinical trial design and management with scientific, statistics and safety review, where we can reduce the time and cost required to conduct patient trials. Equally important is our access to a geographically disperse population, which is especially desirable when conducting gene delivery trials for limited-population neuromuscular disorders.

Successful treatment of severe osteogenesis imperfecta with oral alendronate. *M.E. Miller¹, T.N. Hangartner².* 1) Department of Pediatrics and; 2) BioMedical Imaging Laboratory, Wright State University School of Medicine.

Objective: The purpose of this study was to determine if oral alendronate, a bisphosphonate drug, is effective in treating severe osteogenesis imperfecta (OI). At the request of the parents of a 6 year old boy with severe OI who had recurrent long bone fractures requiring rodding procedures, we began treatment with oral alendronate and followed his clinical response with computed tomography (CT) bone density measurements and a functional assessment tool.

Background: Recent studies have shown that intravenous bisphosphonates are effective in treating severe forms of OI and that bone density measured by CT may be a sensitive enough method to measure the efficacy of treatment modalities in OI. CT bone density measures both a cortical bone density (CBD) and a trabecular bone density (TBD).

We wanted to determine if oral alendronate was effective in treating severe forms of OI. **Methods:** The 6 year old boy was begun on oral alendronate, 5 mg/day for 9 months. Prior to therapy and every 3 months thereafter, a functional assessment [Bleck score, Clin Orthop 159:111-122] and CT bone density were performed. The higher the Bleck score, the greater the function. **Results:** The results are shown in the Table. **Conclusions:** The 30% increase in TBD after 3 months and marked improvement in the Bleck score over 9 months suggest a beneficial effect of the oral alendronate. There were no adverse effects of the treatment. It is not clear why the CBD and TBD began decreasing after 3 months.

	Pre-treatment	3 months	6 months	9 months
TBD (g/cm3)	.100	.130	.110	.105
CBD (g/cm3)	1.70	1.70	1.55	1.42
Bleck score	5	8	12	12

Pharmacological induction of mitochondrial activity corrects the peroxisomal biochemical defect in X-linked adrenoleukodystrophy. *M.C. McGuinness, H.-P. Zhang, J.-F. Lu, S. Kemp, K.D. Smith.* Kennedy Krieger Institute and Johns Hopkins University School of Medicine, Baltimore, MD.

X-linked adrenoleukodystrophy (X-ALD: McKusick 300100) is an inherited neurometabolic disease that has been associated with a deficiency in peroxisomal β -oxidation of very long chain fatty acids (VLCFA). X-ALD maps to Xq28 and the gene encodes a peroxisomal membrane ABC half-transporter protein of unknown function. Previously, we showed that 4-phenylbutyrate (4-PBA) treatment of fibroblasts from X-ALD patients and X-ALD mice resulted in increased peroxisomal VLCFA β -oxidation. Correction of the biochemical defect in X-ALD by 4-PBA was attributed to increased expression of the adrenoleukodystrophy-related gene (ALDR). Identification of more effective pharmacological therapies for X-ALD is necessary, however, because (1) 4-PBA has a short (1-2 h) half-life *in vivo*, and (2) in mice 4-PBA reduced C24 and C26 levels in the brain and adrenal gland to normal levels but after 8 months of continuous treatment levels had returned to pretreatment levels. In this study, we examine the effect of various pharmacological agents (including styrylacetate, benzyloxyacetate, isobutyrate, hydroxybutyrate, trichostatin A, hydroxyurea, 5-azacytidine and lovastatin) on peroxisomal VLCFA β -oxidation in fibroblasts from X-ALD patients and X-ALD mice. We show that styrylacetate and benzyloxyacetate (compounds closely related to 4-PBA) and trichostatin A (a known inhibitor of histone deacetylase) also correct the peroxisomal VLCFA β -oxidation defect. Treatment of X-ALD fibroblasts with 4-PBA, styrylacetate, benzyloxyacetate and trichostatin A initially induces mitochondrial long chain fatty acid (C16) β -oxidation activity resulting in increased peroxisomal VLCFA β -oxidation activity. We show that this increase in peroxisomal VLCFA β -oxidation activity is clearly independent of an increase in expression of ALDR. These studies may contribute to a better understanding of the role of ALDR in peroxisomal VLCFA β -oxidation and suggest that therapies directed towards increasing mitochondrial activity may be beneficial in X-ALD.

Aminoglycoside suppresses a nonsense mutation of the Cu,Zn superoxide dismutase gene in amyotrophic lateral sclerosis. *M. Hirano, W.-Y. Hung, N. Cole, A.C. Azim, H.-X. Deng, T. Siddique.* Neurology, Northwestern University, Chicago, IL.

Cu,Zn superoxide dismutase (SOD1) mutations cause motor neuron degeneration in autosomal dominant amyotrophic lateral sclerosis (ALS). The mechanism of the neurodegeneration is thought to be a toxic gain of function of mutant SOD1 rather than a loss of function of the protein. Although the exact toxic property of the mutant protein remains unknown, release of copper ions or generation of free radicals has been suggested as a trigger of either apoptotic or non-apoptotic cell death. Therapeutic efforts have been aimed at prevention from such possible toxicities or at block of the apoptotic pathway. However, reducing mutant protein synthesis may be an alternative therapeutic option.

Aminoglycosides have been reported to promote suppression of premature stop codons in several mutated genes including the cystic fibrosis conductance regulator gene. This suppression leads to the full-length protein synthesis. We found a nonsense mutation at Leu126 residue of SOD1 in an ALS family, where the mutant protein is predicted to be truncated at the C-terminus. To test possible conversion of the truncated protein to a full-length SOD1 with aminoglycoside agent, we treated cultured transgenic mouse fibroblasts expressing the C-terminal truncated SOD1 protein with 0.1-0.8 mg/ml of gentamicin for 10 days. Immunoblot studies detected bands with the same size as wild-type human SOD1 in the samples after treatments, but not in non-treated fibroblasts. This may indicate that aminoglycoside suppresses the nonsense codon in the gene for SOD1, and make a full-length SOD1 protein in the fibroblasts. This result also provides a possibility to treat transgenic mice and patients expressing this mutant SOD1.

Generation and activity of antibody-targeted FANCF fusion protein in Fanconi anemia group F cells. K.

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The phenotype of Fanconi anemia (FA) includes sensitivity to crosslinkers and bone marrow failure. We previously explored the role of receptor-mediated protein replacement in a cell culture model of FA group C using cytokine ligand-receptor pairs. To test the versatility and improve the efficiency of this approach, here we used an antibody-directed fusion protein to correct the phenotypic defect of a cell line belonging to FA group F. We expressed a 69 kDa chimeric protein (His-CD33-FANCF) in *E. coli* consisting of a histidine (His) tag, a single-chain antibody to the myeloid antigen CD33, and the FA group F (FANCF) protein, as well as a 43 kDa His-FANCF fusion protein lacking the antibody motif. After purification by nickel-agarose chromatography, we used dual-color immunofluorescence to analyze the intracellular trafficking of CD33 and the fusion proteins. In 293 cells transiently transfected with CD33, His-CD33-FANCF bound specifically to the surface of CD33-positive but not to CD33-negative cells, and the complexes internalized in vesicular structures resembling endosomes. The fusion protein, but not CD33, sorted to the nucleus, consistent with the known nuclear location of FANCF. No similar binding or internalization was apparent with His-FANCF. To test the intracellular function of His-CD33-FANCF, the EUFA698 FA group F lymphoblastoid cells were stably transfected with CD33 and exposed to mitomycin C in the presence or absence of 0.1 mg/ml fusion protein. CD33-positive cells treated with the fusion protein were significantly more resistant to the cytotoxic effect of mitomycin C than either untreated cells or parental, CD33-negative cells. Exposure of the CD33-positive EUFA698 cells to His-FANCF did not modify the sensitivity to mitomycin C, indicating the importance of the CD33 epitope. These results demonstrate that antibody-directed receptor-mediated protein replacement is a potentially versatile strategy for the delivery of biologically active proteins into hematopoietic cells.

Therapeutic implications by upregulating SMN2 expression in spinal muscular atrophy. *P. Burlet, S. Bertrand, E. Vial, L. Viollet, A. Munnich, S. Lefebvre.* INSERM U393, IFREM, Hopital Necker Enfant, Paris, France.

The Survival Motor Neuron (SMN1) gene, the disease causing gene for spinal muscular atrophy (SMA), is duplicated with a highly homologous copy gene (SMN2). SMN1 is absent in the majority of patients (>95%) and the remaining patients carry intragenic mutations. A tight correlation between clinical severity and SMN2 protein level was shown. The difference in the level of protein encoded by SMN1 or SMN2 could be due to the promoter activity that regulate the expression of SMN1 and SMN2. SMN1 and SMN2 minimal promoters are identical and contain binding sites for several transcription factors. Transcription factors upregulate the expression of genes by binding to the responsive elements present in the promoters or they downregulate the expression by antagonizing the enhancer action of other transcription factors. To get further insights into the molecular basis underlying SMA and why SMN2 acts as a major modifier gene, it is important to characterize the signaling pathway(s) for SMN1/SMN2 gene expression. One can possibly test a large number of inducers in different experimental conditions and different cell types. Our project is to develop an assay to rapidly analyse the SMN2 protein level that could be scaled up for a high throughput screen of pharmacological molecules.

Gene therapy with adeno-associated virus (AAV) vectors in canine glycogen storage disease, type Ia. *R.M. Beatty¹, M. Jackson², P.S. Kishnani¹, E. Faulkner¹, S. VanCamp², T. Brown², A. Boney¹, Y.T. Chen¹, D.D. Koeberl¹.* 1) Dept Medical Genetics, Duke Univ Medical Ctr, Durham, NC; 2) College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

Current, nutritional therapy in glycogen storage disease type Ia (GSD Ia) does not appear to adequately prevent long-term sequelae, including renal dysfunction, gout, and hepatic adenomas. Thus, we have developed AAV vectors encoding glucose-6-phosphatase (G6Pase) as an approach to gene therapy in GSD Ia. We recently described a canine model for GSD Ia, and it resembles severe GSD Ia in humans. Affected puppies presented with lethargy and weakness, and developed growth delay prior to an early demise. Laboratory evaluation revealed hypoglycemia, lactic acidosis, hyperlipidemia, and elevated uric acid. Postmortem examination revealed hepatomegaly, nephromegaly, hepatic glycogen accumulation, and deficient G6Pase in liver. We have constructed an AAV vector encoding G6Pase driven by the mouse albumin promoter/enhancer (AVALbG6PGH), and administered AVALbG6PGH to an affected puppy on day 3 of life. The puppy had hypoglycemia with blood glucose 21 mg/dl in response to fasting prior to administration of the AAV vector. Four weeks following vector administration fasting glucose was normal at 77 mg/dl. Increased growth for this puppy relative to other affected puppies was observed by 4 weeks of age. Unfortunately, this puppy was orphaned by its mother at 4 weeks of age, and was euthanized at 5 weeks old. Liver G6Pase expression was equivalent to that for a carrier puppy, approximately 50 percent of normal levels. Liver glycogen content was not significantly decreased compared to other affected puppies. Further analysis will include vector DNA analysis in the liver. Initial results indicate that AAV vector-mediated gene therapy in canine GSD Ia could provide a needed, new approach to therapy for this disorder.

Gene replacement therapy of citrullinemia using helper-dependent adenoviral vectors. *B. Mull, A. Mian, L. Pastore, G. Toietta, O. Bodamer, A.L. Beaudet, B. Lee.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Citrullinemia is an autosomal recessive disorder caused by a deficiency of human argininosuccinate synthetase (hASS), a required enzyme of the urea cycle. Insufficient hASS activity prevents the efficient removal of ammonia derived from amino acid metabolism. We have developed an adenoviral vector containing the hASS cDNA expression cassette in a helper dependent vector system, in which the adenoviral construct is completely devoid of all viral genes (AdDBOShASS). Helper dependent vectors (HDV) have been shown to exhibit prolonged transgene expression without the acute and chronic toxicity associated with first generation, E1-deleted adenoviral vectors. Novel methods for titering these vectors have been developed as traditional plaque assay protocols are not applicable. Southern analysis in conjunction with real time PCR showed helper virus contamination <1% in the HDV (AdDAdDBOShASS). In vitro, ASS-deficient XC cells expressed de novo ASS activity after infection with AdDAdDBOShASS. In vivo, both first generation adenoviral vector (AdDE1CAGhASS) and the HDV (AdDAdDBOShASS) treated mice expressed high levels of hASS mRNA at day 3, but only the HDV treated mice continued to express hASS at 8 weeks after injection. Moreover, only mice treated with the first generation vector showed signs of hepatotoxicity as evidenced by elevated liver function tests. These data suggest that the increased therapeutic index afforded by HDV will allow for their use in diseases, such as the urea cycle disorders, which require efficient hepatocyte transduction and high level gene expression.