Program Nr: 1 from the 2001 ASHG Annual Meeting

A novel forkhead-domain gene is mutated in a severe speech and language disorder. *S.E. Fisher[1], C.S.L. Lai[1], J.A. Hurst[2], F. Vargha-Khadem[3], A.P. Monaco[1].* 1) Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, UK; 2) Dept. of Clinical Genetics, Oxford Radcliffe Hospital, Oxford, UK; 3) Institute of Child Health, London, UK.

Individuals affected with developmental disorders of speech and language have substantial difficulty acquiring expressive and/or receptive language despite adequate intelligence and opportunity, and in the absence of any profound sensory or neurological impairment. Although twin studies consistently demonstrate a significant genetic component, the majority of families segregating speech and language deficits show complex patterns of inheritance, and no gene predisposing to such disorders has previously been identified. We studied a unique three-generation pedigree, KE, in which a severe speech and language disorder is transmitted as an autosomal-dominant monogenic trait (MIM 602081). Our earlier work mapped the locus responsible (SPCH1) to a 5.6cM interval of 7q31. Bioinformatic analysis of genomic sequence data enabled us to construct a comprehensive transcript map of the SPCH1 locus. Here we report the isolation of a novel gene from this interval which encodes a new member of the forkhead/winged-helix family of transcription factors. We discovered that this gene is directly disrupted by a translocation breakpoint in an unrelated patient who has a language disorder that is strikingly similar to that of the KE family. Moreover, we identified a point mutation in all affected members of the KE family, which alters an invariant amino-acid residue in the DNA-binding domain encoded by the gene. We propose that haploinsufficiency of this forkhead gene during embryogenesis leads to abnormal development of neural structures that are important for speech and language. This is the first gene to have been implicated in such pathways and will offer novel insights into the molecular processes mediating this uniquely human trait.

Program Nr: 2 from the 2001 ASHG Annual Meeting

Cystic fibrosis prenatal screening of 27,000 women in a large HMO. *D.R. Witt, J. Coppinger.* Genetics Dept, Kaiser Permanente, San Jose, CA.

This presentation gives an update on the findings from the first two years of operation of a large CF prenatal screening program in the Kaiser Permanente Medical Care Program of Northern California. The report will also review some of the salient planning and implementation issues. This will include the efficient integration of CF testing with existing screening for other diseases, staff training, development of effective pre-test educational materials and tracking/reporting systems for patients and specimens, and molecular laboratory requirements for high volume specimen throughput.

The Kaiser program offers prenatal screening in a sequential format (female partner tested first; male partner tested only if the woman identified as a carrier) to couples in which at least one partner is Caucasian. Acceptance of testing is high; approximately 27,000 women have been screened and 1,000 carriers identified with an overall carrier identification rate of 1/28. Data will be presented on 24 high-risk couples and prenatal diagnosis outcomes. Screening is done by a panel of 37 CFTR mutations and the poly T variant; 30 different mutations have been detected at least once. Interesting results include the very high incidence of the R117H mutation with associated poly T variants and mild mutations in general, counseling issues related to genotype-phenotype correlation, compound heterozygosity in asymptomatic women, and comparison of molecular results in couples in whom both partners are Caucasian vs. "mixed" couples. It is hoped that data from this program will assist other institutions and providers in the implementation of CF screening and guide decision-making for issues such as pre-test education, eligibility criteria for testing based on ethnicity, and mutation panel composition.

.

Program Nr: 3 from the 2001 ASHG Annual Meeting

Analysis of type I procollagen synthesized by cultured fibroblasts in evaluation of children with fractures thought to be due to non-accidental injury. A.B. Marlowe, M.G. Pepin, P.H. Byers, University of Washington, Seattle.

A.B. Marlowe[1], M.G. Pepin[2], P.H. Byers[2]. 1) Biobehavioral Nursing, Univ Washington, Seattle, WA; 2) Pathology, Univ Washington, Seattle, WA.

A child with multiple fractures frequently raises the question of non-accidental injury (NAI). Because children with OI may have undetected bone breaks, the finding of fractures in varying stages of repair has, at times, led to accusations of child abuse in OI families. The availability of testing for OI has led some accused of NAI to claim that their child has OI, and that fractures were a consequence of the disorder. OI usually results from dominant mutations in one of the two genes that encode the chains of type I procollagen. The genes are expressed in dermal fibroblasts and 85-90% of individuals with OI can be indentified by studies of collagens synthesized by those cells. From 1998-2000, our laboratory received 262 samples (out of 3449) from children in whom fractures raised the suspicion of NAI. We identified abnormalities in 11 of 262 samples. Studies were indeterminant in 11 instances while cells from the remaining 240 children made normal amounts of apparently normal type I procollagen. Of 262 samples 137 were accompanied by adequate clinical history. In 7 of these 137 samples we identified abnormalities: 4 with decreased synthesis of type I procollagen compatible with OI type I and 3 with cells that synthesized normal and abnormal collagen molecules conpatible with OI type III or IV; 7 studies were equivocal. In a retrospective review, in 6 of 7 children with abnormal biochemical studies, the referring physician "suspected OI". Five of the 7 had two signs in addition to fractures (e.g blue-grey sclerae, Wormian bones, bowing of long bones, osteopenia or a close relative with fractures). Among the 123 with normal studies and adequate information, 60% of children had one or more sign of OI and 14% had two or more yet the referring physician seldom suspected OI, indicating that these are not good diagnostic indicators in infants. In sum, testing for OI is valuable to confirm a suspected diagnosis, however, biochemical confirmation is very low if the diagnosis of OI is believed unlikely.

Program Nr: 4 from the 2001 ASHG Annual Meeting

Genetic model of multi-step breast carcinogenesis involving the epithelium and stroma: clues to tumor-microenvironment interactions. *K. Kurose[1], S. Hoshaw-Woodard[2], A. Adeyinka[3], S. Lemeshow[2], P. Watson[3], C. Eng[1,4]*. 1) Clinical Cancer Genetics and Human Cancer Genetics Programs, The Ohio State University, Columbus, OH; 2) Center for Biostatistics, The Ohio State University, Columbus, OH; 3) Department of Pathology, University of Manitoba Health Sciences Center, Winnipeg, Manitoba, Canada; 4) CRC Human Cancer Genetics Research Group, University of Cambridge, Cambridge, UK.

Although numerous studies have reported that high frequencies of loss of heterozygosity (LOH) at various chromosomal arms have been identified in breast cancer, differential LOH in the neoplastic epithelial and surrounding stromal compartments has not been well examined. Using laser capture microdissection, which enables separation of neoplastic epithelium from surrounding stroma, we microdissected each compartment of 41 sporadic invasive adenocarcinomas of the breast. Frequent LOH was identified in both neoplastic epithelial and/or stromal compartments, ranging from 25% to 69% in the neoplastic epithelial cells, and from 17% to 61% in the surrounding stromal cells, respectively. The great majority of markers showed a higher frequency of LOH in the neoplastic epithelial compartment than that in the stroma, suggesting that LOH in neoplastic epithelial cells might precede LOH in surrounding stromal cells. Furthermore, we sought to examine pair-wise associations of particular genetic alterations in either epithelial or stromal compartments. Seventeen pairs of markers showed statistically significant associations. We also propose a genetic model of multi-step carcinogenesis for the breast involving the epithelial and stromal compartments and note that genetic alterations occur in the epithelial compartments as the earlier steps followed by LOH in the stromal compartments. Our study strongly suggests that interactions between breast epithelial and stromal compartments might play a critical role in breast carcinogenesis and several genetic alterations in both epithelial and stromal compartments are required for breast tumor growth and progression. A total genome LOH scan of epithelial and stromal compartments is on-going.

Program Nr: 5 from the 2001 ASHG Annual Meeting

Development and validation of a high-resolution genomic microarray for identifying constitutional chromosome abnormalities. *M.S. Mohammed[1], B.A. Bejjani[1], S. Shah[2], J.R. Lupski[1], L.G. Shaffer[1]*. 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Spectral Genomics, Houston, TX.

CGH to metaphase chromosomes is limited to detecting chromosomal aberrations greater than ~ 5 Mb (<650 band resolution). A modified CGH to BACs immobilized on a glass slide (instead of metaphase chromosomes) can provide a much higher resolution, potentially resolving to the size of individual BACs. We developed a human genomic array in which BACs are spaced at ~ 3 Mb intervals, on average, throughout the genome. In addition, a higher density of BACs is arrayed in clinically significant genomic regions that include the telomeres and known microdeletion syndromes. Clinical specimens and established cell lines with a broad spectrum of known chromosomal abnormalities were tested. Test and reference genomic DNAs were differentially labeled with fluorochromes. After co-hybridization of labeled test and reference genomic DNAs, the BAC arrays were scanned and analyzed with software developed specifically for this purpose. Analysis was performed in a blinded fashion for the majority of cases. Control hybridizations with normal male-to-male, male-to-female and female-to-female reference samples were performed and showed the expected results. The unbalanced chromosomal aberrations included partial chromosome arm gains/losses; cytogenetically detectable small deletions, including monosomy 1p36; cryptic deletions identified by FISH, but not by routine G-banding, including deletions of 4p16.3, 11p11.2 and 22q11.2; and double segmental imbalances, including a case with both deletion of 1p36 and duplication of 9q34. All chromosomal aberrations, including those from the blinded samples, were identified correctly. These results demonstrate the reliability of this technique in detecting 2:1 and 3:2 segmental imbalance. The current array is equivalent to over 1,000 individual FISH experiments supporting that array CGH will likely develop into the method of choice for rapid genomic screening in genetic diagnostics. BAC arrays will prove useful for the detection of constitutional aneusomies including those that are cryptic and otherwise undetectable even by high-resolution cytogenetic analyses.

Program Nr: 6 from the 2001 ASHG Annual Meeting

The extent and diversity of common human haplotypes. *S. Bolk[1], J. Higgins[1], J. Moore[1], H. Nguyen[1], J. Roy[1], S. Schaffner[1], E.S. Lander[1,2], M.J. Daly[1], D. Altshuler[1,3].* 1) Center for Genome Research, Whitehead Inst, MIT, Cambridge, MA; 2) Department of Biology, MIT; 3) Massachusetts General Hospital and Harvard Medical School, Boston, MA.

Haplotype-based association studies offer a powerful approach to disease gene mapping, based on linkage disequilibrium (LD) between disease-causing variants and markers on the ancestral haplotypes on which they arose. The generality of the approach depends on characteristics of disease causing alleles and underlying haplotype structure. We are characterizing haplotypes and LD by genotyping common SNPs in CEPH (93 individuals). Spanning a total of 7.5 Mb, with average marker spacing of 4.5kb, the first stage of our study represents one of the most detailed and extensive descriptions of LD and haplotype structure across the genome. We generated 400,000 genotypes for a total of 4,100 SNPs distributed in fifty 150 kb regions evenly-spaced across the genome. All SNPs have been selected from the publicaly available SNP map generated by TSC and BAC overlap projects.

We have performed a preliminary analysis limited only to common SNPs (>20%), and observe extensive linkage disequilibrium in most regions. We find that 80% of pairs show complete LD at short distances (<3kb), and 50% of pairs retain D'=1 up to 20kb. Mean values of D' remain >0.5 at distances up to ~60-80kb. Interestingly, we observe discrete blocks of strong LD (adjacent marker pairs showing statistically significant LD) juxtaposed with regions lacking LD. The blocks range from 5 to 150kb (our upper bound) and are separated by apparent sites of recombination. Finally, since genotyping was performed in families, we are able to analyze fully-phased haplotypes across each regions. The block-like LD patterns are attributable to a limited number of common haplotypes with substantial recombination between them. Our results indicate that even very common alleles show extensive LD and are associated with limited haplotype diversity. These results suggest that LD mapping will be of general utility in populations similar in characteristics to CEPH. We will test these regions in additional populations to determine the generality of the patterns.

Program Nr: 7 from the 2001 ASHG Annual Meeting

Abnormal brain structure and function in subjects with Van der Woude Syndrome. *P. Nopoulos[1], B. Schutte[2], S. Daack-Hirsch[2], J. Nichols[1], J. Murray[2].* 1) Department of Psychiatry, University of Iowa, Iowa City, IA; 2) Department of Pediatrics, University of Iowa, Iowa City, IA.

Objective: In a previous study of adults with non-syndromic clefts of the lip and palate, significant abnormalities in brain structure and function were identified. Van der Woude Syndrome (VDWS) is an autosomal dominant disorder that manifests a phenotype very similar to non-syndromic cleft lip/palate. The aim of this study was to determine if subjects with VDWS also have abnormal brain morphology and/or cognitive dysfunction. Brain morphology (using quantitative MRI) and performance on cognitive tasks in a sample of 12 adults with VDWS were analyzed and compared to 12 healthy control adults matched for sex and age.

Results: As a group, the subjects with VDWS had significantly lower Intelligence Quotients (IQ) compared to their matched controls. In particular, there were sex-specific patterns with male VDWS subjects having more deficits in language areas and females with VDWS having greater deficits in non-verbal skills. In regard to brain morphology, VDWS subjects had significantly *larger* than normal frontal and parietal lobe volumes. This was accounted for by an increase in the gray matter volume of these regions. Subcortical gray and white matter volumes were significantly *smaller* in subjects with VDWS. Correlations between brain structure and function showed direct relationships between abnormalities in brain volume and cognitive deficits.

Conclusion: Subjects with VDWS have significant abnormalities in brain structure and function. In particular, they manifest a pattern of abnormalities very similar to that of adult subjects with non-syndromic clefts of the lip and palate: increase in the volume of gray matter in the anterior region of the cerebrum. Unique to VDWS was volume deficit in subcortical structures. Cognitive deficits as well as abnormality in brain structure manifested sex-specific patterns. The abnormal brain structure is most likely due to aberrant brain development. This study highlights the complex interaction and interdependence of craniofacial and cerebral development.

Program Nr: 8 from the 2001 ASHG Annual Meeting

Neuropathology and neurodegenerative features in some older male premutation carriers of fragile X syndrome.

R.J. Hagerman[1], C. Greco[1,2], A. Chudley[3], M. Leehey[4], F. Tassone[5], J. Grigsby[6], J. Hills[7], R. Wilson[7], S.W. Harris[1], P.J. Hagerman[5]. 1) M.I.N.D. Institute, UC Davis Medical Center, Sacramento, CA; 2) Department of Pathology, UC Davis Medical Center, Sacramento, CA; 3) Department of Pediatrics, University of Manitoba, Canada; 4) Department of Neurology, University of Colorado Health Sciences Center, Denver, CO; 5) Department of Biological Chemistry, UC Davis, Davis, CA; 6) Department of Medicine, Univeristy of Colorado Health Sciences Center, Denver, CO; 7) Child Development Unit, The Children's Hospital, Denver, CO.

Eleven males who are premutation carriers and grandfathers of children with fragile X have been seen with a progressive neurodegenerative syndrome characterized by a constellation of features, including intention tremor, executive function and memory deficits, parkinsonian features, decreased sensation in the lower extremities, ataxia and frequent falling, and difficulties with activities of daily living, such as handwriting, dressing and eating independently. The clinical course usually begins in the 50's or 60's and is slowly progressive over a 10 to 15 year period. MRI studies in eight individuals have shown generalized brain atrophy. Molecular studies have demonstrated elevated FMR1 mRNA at levels of 2 to 4 times normal. Neuropathology after death in two patients has revealed spherical eosinophilic intranuclear inclusion bodies in neurons and glial cells throughout the brain. The cause of the intranuclear inclusions is unknown and we are currently investigating a possible role of elevated mRNA in these inclusions. Only a small subgroup of older males with the premutation appears to be affected with this neurodegenerative process and the overall prevalence is under investigation in Davis, Denver, Chicago, England, and Australia. Involvement with this neurodegenerative syndrome may be related to a second gene mutation interacting with the FMR1 premutation.

Program Nr: 9 from the 2001 ASHG Annual Meeting

A study of 220 patients with polymicrogyria delineates distinct phenotypes and reveals multiple genetic loci. *R.J. Leventer[1], C.M. Lese[1], C. Cardoso[1], J.A. Roseberry[1], A. Weiss[1], N. Stoodley[2], D.T. Pilz[2], L. Villard[3], K. Nguyen[3], G.D. Clark[4], H.A. Heilstedt[4], L.G. Shaffer[4], R. Guerrini[5], W.B. Dobyns[1]*. 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) University Hospital of Wales, Cardiff, UK; 3) Inserm U491, Marseille, France; 4) Baylor College of Medicine, Houston, TX; 5) Institute for Child Health, London, UK.

Magnetic resonance imaging has facilitated the identification of a spectrum of cortical malformations and delineation of the genetic and developmental basis of these disorders will advance knowledge of normal brain development. One of the most common malformations is polymicrogyria (PMG), which is associated with epilespy, mental retardation, speech dysfunction and spasticity. PMG is characterized by excessive gyration and a disorganized cortical architecture, and shows a predilection for the Sylvian fissures. It usually occurs as an isolated birth defect. Our aims were to define the clinical and imaging features, the relative frequencies of subtypes and to summarize new data to support a genetic etiology in a large cohort of patients with PMG. We identified at least 8 distinct PMG subtypes of which the bilateral perisylvian distribution was most common (60%). There was a frequent association with other brain malformations, including abnormalities of the corpus callosum (32%), white matter (31%) and cerebellum (14%). Males were affected more than females (p=0.019). We identified 12 pedigrees with multiple affected members, of which 5 were compatible with XL inheritance, 4 with AR inheritance, and 3 with either inheritance pattern. Consanguinity was present in 2 families. 9 unrelated patients had structural chromosomal anomalies leading to haploinsufficiency involving chromosomes 1p36, 2p13, 6q25, 21q22 and 22q11, and linkage mapping in 5 families with an XL inheritance pattern mapped to distal Xq (Villard et al., this meeting). Our data strongly support a genetic etiology for PMG, with multiple genetic loci associated with the most common perisylvian phenotype. This work forms the basis for studies to identify the causative genes for PMG and we hypothesize that these proteins interact in common or related pathways for cortical development.

Program Nr: 10 from the 2001 ASHG Annual Meeting

Polymorphic genomic mutation on human chromosome 15 and susceptibility to anxiety disorders (panic disorder and social phobia). *M. Gratacos[1], M. Nadal[1], R. Martin-Santos[2], M.A. Pujana[1], J. Gago[2], B. Peral[1], Ll. Armengol[1], I. Ponsa[3], R. Miro[3], A. Bulbena[2], X. Estivill[1].* 1) Medical and Molecular Genetics Center, Barcelona, Spain; 2) Psychiatry Department, Hospital del Mar, IMAS, Barcelona, Spain; 3) Cellular Biology Department, UAB, Barcelona, Spain.

Anxiety disorders are heterogeneous, complex and common psychiatric conditions that include panic disorder, agoraphobia, social phobia and simple phobia. We have studied the molecular basis of the co-occurrence of panic and phobic disorders with joint laxity and have identified an interstitial duplication of human chromosome 15q (DUP25). DUP25 is flanked by several copies of a new chromosome 15 specific duplicon (named LCR15), which is part of several sets of segmental duplications of this chromosome. Panic disorder, agoraphobia, social phobia and/or joint laxity gave significant association between DUP25 and the psychiatric disorders and joint laxity in families, using association ($P = 0.04$ to $P = 0.0001$), parametric (lod score 5.0 at a $q = 0$) and non-parametric ($P = 0.00006$) methods. DUP25 was also detected in 68 of 70 unrelated cases of panic disorder, but in only 14 of 189 control subjects ($P < 0.0001$). The absence of linkage between 15q markers and DUP25 and the psychiatric phenotypes, the presence of mosaicism in subjects with DUP25, and the detection of different forms of the duplication within the same family, suggest that DUP25 may be a new non-Mendelian mechanism of disease causing mutation and raises the possibility that another locus causes DUP25 (paramutagenic locus) or that specific configurations of duplicons are particularly susceptible to undergo duplication (paramutable locus), leading to DUP25. The DUP25 mechanism that leads to panic disorder and joint laxity is probably through a dosage effect, with overexpression of one or several genes of the 15q region. Since DUP25 is present in 7% of the general population, it should be considered a major factor for susceptibility to anxiety disorders. Supported by La Marato de TV3 and the Spanish Ministry of Science and Technology.q.

Program Nr: 11 from the 2001 ASHG Annual Meeting

Large scale deletion and SMADIP1 truncating mutations in syndromic Hirschsprung disease with midline structure involvement. *J. Amiel[1], Y. Espinosa-Parrilla[1], J. Steffann[1], A. Pelet[1], P. Gosset[1], A. Choiset[2], H. Tanaka[3], M. Prieur[1], M. Vekemans[1], A. Munnich[1], S. Lyonnet[1]*. 1) Department of Genetics and INSERM U-393, Necker-Enfants Malades Hospital, Paris; 2) Cytogenetics, Saint Vincent de Paul Hospital, Paris, France; 3) Department of Pediatrics, Asahikawa, Japan.

Hirschsprung disease (HSCR) is a common malformation of neural crest-derived enteric neurons that is frequently associated with other congenital abnormalities. The SMADIP1 gene has been recently recognized as disease causing in some patients with 2q22 chromosomal rearrangement, resulting in syndromic HSCR with mental retardation, microcephaly and facial dysmorphism. A total of 19 cases among a series of 250 HSCR patients were selected on the following criteria: i) normal chromosome analysis on standard examination, ii) moderate to severe mental retardation, and, iii) one of the following features: facial dysmorphism, cerebral anomaly including seizures, microcephaly, or agenesis of the corpus callosum. The SMADIP1 locus was screened by high resolution chromosomal analysis, poly (CA) microsatellite markers flanking SMADIP1 and SSCP of the gene coding sequence. An interstitial deletion of chromosome 2q22 was observed on high resolution karyotype in one case. Loss of heterozygosity at the SMADIP1 locus allowed to identify a submicroscopic de novo deletion in 3 further cases. Finally, Nucleotidic variations were identified in 4 cases (L562X, 935 del G, 1805 del A, and 2453 ins T). We therefore identified large-scale SMADIP1 deletions or truncating mutations in 8/19 cases. These results allow to further delineate the spectrum of malformations ascribed to SMADIP1 haploinsufficiency by adding congenital defects of cardiac septation, agenesis of the corpus callosum and hypospadias as frequent features in this syndrome. Conversely, the MCA-MR syndrome referred to as Goldberg-Shprintzen syndrome (HSCR, mental retardation and cleft palate, MIM 235730) represents a different condition. Finally, our findings suggest that Smadip1 is not only involved in neural crest-derived cells and central nervous system patterning, but also in heart septation and development of midline structures.

Program Nr: 12 from the 2001 ASHG Annual Meeting

Polyalanine tract contraction and missense mutation of the h-ASH1 gene in Congenital Central Hypoventilation Syndrome (CCHS, Ondine's curse). *L. de Pontual[1], T. Attie-Bitach[1], H. Trang[2], C. Pajot[1], M. Simonneau[2], M. Vekemans[1], A. Munnich[1], S. Lyonnet[1], C. Gaultier[2], J. Amiel[1]*. 1) Department of Genetics and INSERM U-393, Necker-Enfants Malades Hospital; 2) Physiology Unit, INSERM E9935, Robert Debre Hospital.

CCHS is a hitherto unexplained disorder of the metabolic control of breathing. CCHS is associated with Hirschsprung disease in 25% of cases (Haddad syndrome, MIM 209880), suggesting a common defect of neural crest derived cells. Thus far, only rare mutations of the RET-GDNF and the endothelin pathways have been reported. The h-ASH 1 gene encodes a neural-specific bHLH transcription factor that contains both polyalanine and polyglutamine stretches. h-ASH 1 has been regarded as a candidate gene in CCHS due to: i) its role in early neuronal differentiation upstream to the RET-GDNF pathway, ii) the phenotype of homozygous knock-out mice, and iii) its expression in the central (CNS) and peripheral nervous systems (PNS) of mouse embryo. In this study, 30 patients were screened for h-ASH 1 gene mutations, by SSCP and direct DNA sequencing (26 isolated CCHS patients and 4 Haddad syndrome cases). We identified 2 heterozygous polyalanine tract contractions of 5/13 and 8/13 codons respectively, as well as one missense mutation (P18T). These DNA variations were not found in 180 control chromosomes. In addition, 3 h-ASH 1 gene polymorphisms were found with similar allele frequencies as compared to controls (V215V, CAG repeat size, and -137 T>C). In order to further support the involvement of H-ASH 1 in CCHS, in situ hybridization was performed in human embryo from 5 to 9 weeks, using a h-ASH 1 222 bp riboprobe generated by PCR. This study showed h-ASH 1 expression in the neural tube by C14 (d33), CNS and para aortic ganglia by C16 (d37) and adrenal glands and hindgut by C18 (d44). Although polyalanine expansion is well documented in human, contraction has not been hitherto reported. In vitro studies are underway to investigate the putative role of these mutations on h-ASH 1 function. Finally, these findings should support the view of polygenic inheritance of CCHS involving genes from the RET-GDNF signaling pathway.

Program Nr: 13 from the 2001 ASHG Annual Meeting

Novel insights regarding the pathogenesis of cerebral cavernous malformation (CCM). *J. Zhang[1], R.E. Clatterbuck[2], D. Rigamonti[2], D.D. Chang[3], H.C. Dietz[1].* 1) JHMI and Inst of Genetic Med,Johns Hopkins SOM, Baltimore, MD; 2) Dept of Neurosurgery, Johns Hopkins SOM, Baltimore, MD; 3) Dept of Medicine, UCLA SOM, Los Angeles, CA.

CCM is a common autosomal dominant disorder characterized by abnormal venous sinusoids that predispose to intracranial hemorrhage. CCM is genetically heterogeneous, with loci at 7q, 7p, and 3q. Mutations in KRIT1 account for all cases linked to 7q (CCM1), but the pathogenesis of CCM is not understood. Krit1 (Krev Interaction Trapped 1) was originally identified through its interaction with the Ras-family GTPase krev/rap1a in a 2-hybrid screen, inferring a role in GTPase signalling cascades. We demonstrated additional coding exons at the 5'-end of the krit1 coding sequence, extending the N-terminus by 207 amino acids compared to the previously reported protein. Remarkably, by 2-hybrid analysis and co-IP, full-length krit1 fails to interact with krev/rap1a, suggesting that the original observation was spurious. 2-hybrid screening revealed that krit1 interacts with icap1 (integrin cytoplasmic domain-associated protein-1), a result that was confirmed by co-IP. Icap1 binds to a NPXY motif in the cytoplasmic domain of b1 integrin and participates in b1-mediated cell adhesion and migration. The novel N-terminus of krit1 contains a NPXY motif and we show by site-directed mutagenesis that it is required for icap1 interaction. Like b1 integrin, krit1 interacts with the 200aa isoform of icap1 (icap-1A), but not a 150aa form that results from alternative splicing (icap-1B). Mutations in icap-1A that enhance or diminish binding with b1 integrin have concordant effects for krit1, suggesting that the two proteins bind to the same site on icap-1A. These data support a model where krit1 and b1 integrin compete for the same binding domain in icap-1A, perhaps constituting a regulatory mechanism. Loss-of-function KRIT1 mutations, as observed in CCM1, would shift this balance with predicted consequences for endothelial cell performance during integrin b1-dependent angiogenesis. This mechanistic understanding may facilitate identification of other CCM genes and the development of rational therapeutic strategies.

Program Nr: 14 from the 2001 ASHG Annual Meeting

Characterization of dFXR: the fly homologue of FMR1. *J. Morales, B. Hassan, H. Bellen, D.L. Nelson.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Fragile X Syndrome, one of the most common forms of mental retardation is caused by the loss of function of the FMR1 gene. The expansion and hyper methylation of a CGG repeat in the 5 UTR of the gene results in silencing at the locus and loss of expression of FMR1. The protein encoded by the gene, FMRP, is a ubiquitously expressed RNA binding protein thought to be involved in the processes of learning and memory. Though mouse models have been analyzed, much of the basic biology of FMRP has yet to be elucidated. Recently, the completion of the Drosophila Sequencing Project allowed the identification of dFXR, the fly homologue of FMR1. Sequence analysis has revealed an overall conservation of 30% (AA level) and up to 60% in the functional domains. Similar to its vertebrate counterpart, dFXR is an RNA binding protein that displays a ubiquitous pattern of expression. In order to analyze the consequences of dFXR loss, three P-element excision lines as well as the parental insertion line have been characterized. RNA and protein analyses indicate that all excision lines carry null alleles of dFXR. A recessive lethal effect has been documented for all alleles and shown to occur late in development with a variable degree of penetrance. In addition, phenotypes ranging from cell body absence to abnormal axon branching have been observed in the lateral neurons of the brain. These neurons are involved in the regulation of circadian rhythm. The excision lines will be tested for rhythm abnormalities. Over expression studies have also been performed and viability was noted when expression was limited to a subset of cells. An axon guidance phenotype has been observed in atonal cells. These cells form an intricate pattern of axon connections that facilitate the identification of axonal phenotypes. We are currently analyzing these same cells in the loss of function background. Over expression will also be targeted to the lateral neurons. In addition, transgenic flies carrying mouse FMR1 have been generated to determine if protein function is conserved. Experiments are underway to test whether vertebrate FMRP is capable of rescuing the invertebrate phenotypes.

Program Nr: 15 from the 2001 ASHG Annual Meeting

Severe neural tube defects in the loop-tail mouse result from mutation of loopin, a novel transmembrane protein.*loop-tail*. *C. Paternotte[1], J.N. Murdoch[1], K. Doudney[2], P. Stanier[2], A.J. Copp[1]*. 1) Neural Development Unit, Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH, UK; 2) Department of Maternal and Fetal Medicine, Institute of Reproductive and Developmental Biology, Imperial College School of Medicine, Hammersmith campus, London W12 0NN, UK.

Neural tube defects (NTDs) are major congenital malformations whose molecular mechanisms are poorly understood. In order to elucidate the process of closure of the neural tube, we need to identify the genetic mechanisms involved. The mouse mutant loop-tail (Lp) provides a model for the most severe form of NTD, craniorachischisis. Homozygous Lp embryos die at around the time of birth and fail to initiate neural tube closure at embryonic day 8.5 (E8.5), leading to an open neural tube from the midbrain to the tail. Other malformations include eyelid fusion and heart defects. Heterozygotes are viable and fertile despite exhibiting a tail defect, head wobbling behaviour and often imperforate vagina. The Lp gene maps to distal mouse Chromosome 1, in a region corresponding to human 1q22-q23. We have built a complete physical map of the Lp region that comprises YACs, PACs, BACs and P1 clones, covering approximately 3.2 megabases (Mb), and used this to construct an exhaustive human and mouse gene map of the critical interval. Twelve genes map within the critical region and sequence comparison between their coding regions in Lp/Lp and +/+ DNAs has revealed a mutation in one of them. Here we describe the identification of a mutation in a novel gene (which we have termed loopin) in the Lp mouse, providing the first information on the genetics of craniorachischisis. We also present expression data and show that we can detect loopin mRNA in areas correlating with the various defects seen in the Lp mouse. Following the identification of loopin, we have started screening the human homologue for mutations in a set of 150 human NTD samples, whose phenotypes include craniorachischisis, exencephaly and closed and open spina bifida. *loop-tailloopinLpLp/Lp*.

Program Nr: 16 from the 2001 ASHG Annual Meeting

Coilin forms the bridge between Cajal bodies and SMN, the Spinal Muscular Atrophy protein. *A.G. Matera[1,2], K.E. Tucker[1,2], P.W. Szymczyk[1,2], K.B. Shpargel[1,2], M.D. Hebert[1,2].* 1) Dept Genetics, Case Western Reserve Univ, Cleveland, OH; 2) Center for Human Genetics, University Hospitals of Cleveland, Cleveland, OH.

Spinal muscular atrophy (SMA) is a lethal genetic disorder caused by mutations in the human survival motor neurons gene, SMN1. SMN protein is part of a large complex that is required for biogenesis of various small nuclear ribonucleoproteins (snRNPs). In addition to snRNPs, they are highly enriched in basal transcription and cell cycle factors, the nucleolar proteins fibrillarin and Nopp140, the survival motor neurons (SMN) protein complex, and the Cajal body (CB) signature protein, coilin. Here we report the generation of knockout mice lacking the C-terminal 487 aa of coilin. Some homozygous mutant animals are viable, but their numbers are significantly reduced when crossed to inbred backgrounds. Analysis of tissues and cell lines from knockout animals reveals the presence of extranucleolar foci that contain fibrillarin and Nopp140 but not other typical nucleolar markers. These so-called residual CBs neither condense Sm proteins nor recruit members of the SMN protein complex. Transient expression of wildtype mouse coilin in knockout cells results in formation of CBs and restores these missing epitopes. Additional experiments show that SMN interacts directly with coilin, and that this interaction mediates recruitment of the SMN complex to CBs. Mutation of specific residues within coilin inhibits the interaction both in vivo and in vitro. Ectopic expression of SMN and coilin constructs in coilin knockout cells confirms that recruitment of SMN and splicing snRNPs to CBs depends on a C-terminal RG dipeptide motif. A cardinal cellular feature of SMA is a defect in the nuclear targeting of SMN; our results uncover a role for coilin in this process.

Program Nr: 17 from the 2001 ASHG Annual Meeting

Structure and evolution of genomic duplications in 15q11-q13. *D.P. Locke[1], A.M. Yavor[1], J. Lehoczky[2], J. Chang[2], K. Dewar[2], S. Zhao[3], R.D. Nicholls[4], S. Schwartz[1], E.E. Eichler[1].* 1) Department of Genetics, Case Western Reserve University, Cleveland, OH; 2) WI/MIT Center for Genome Research, Cambridge, MA; 3) The Institute for Genomic Research, Rockville, MD; 4) University of Pennsylvania, Philadelphia, PA.

Phase I of the Human Genome Project has revealed the difficulty in assembling regions harboring genomic duplications by sequence similarity-based methods. Therefore, specialized approaches are required to accurately sequence and assemble these duplicated regions. Using 15q11-q13 as a prototype, we are developing techniques to ensure proper sequence coverage in duplication-rich areas. In addition, we are studying the evolutionary history of the human 15q11-q13 duplication clusters by comparative sequencing in multiple primate species, to gain insight into the mechanisms that gave rise to these structures.

The 15q11-q13 region contains numerous partial duplications of the *HERC2* genomic locus (15q13). The *HERC2* duplications cluster at the Prader-Willi and Angelman syndrome common deletion breakpoints, where paralogous recombination is hypothesized to occur. From available sequence information, we have developed STSs that amplify all known *HERC2* duplicons. Phylogenetic analysis of STS sequences from RPCI-11 *HERC2*-positive clones identified ~10 distinct *HERC2* sequence signatures which will be used to focus sequencing efforts. Furthermore, a subset of *HERC2* duplicons can be unambiguously positioned based on flanking unique sequence. Sequence and FISH analysis of *HERC2* clusters reveals a significantly more complex structure than previously reported. We have found intra- and inter-chromosomal duplications in association with the *HERC2* duplicons, including multiple genic segments. Baboon sequence analysis shows a surprising conservation of intra-chromosomal duplication partners, indicating the relationship between duplicons within the clusters was established prior to the divergence of human and baboon. Also, the pattern of sequence similarity between *HERC2* duplicons suggests that gene conversion may be playing a role in homogenizing these sequences in multiple primate species.

Program Nr: 18 from the 2001 ASHG Annual Meeting

Genomic analysis of 280 kb of DNA sequence from human chromosome 2q13 surrounding an ancestral chromosome fusion site. *Y. Fan, L. Linardopoulou, C. Friedman, B.J. Trask.* Fred Hutchinson Cancer Research Center, Seattle, WA.

Human chromosome 2 was formed by the head-to-head fusion of two ancestral chromosomes that remained separate in other primates. As a result of this fusion, sequences that once resided near the ends of the ancestral chromosomes are now interstitially located in band 2q13. Since portions of these sequences were already duplicated at other subtelomeric locations prior to the fusion, the fates of genes residing in dynamic subtelomeric regions can be compared with that of their paralogs trapped at this interstitial location. To understand the sequence organization, gene content and evolutionary history of this region, we used database mining, PCR assays of a hybrid panel and BACs, sequencing, and FISH to analyze 280 kbp surrounding the fusion site and related sequences on other chromosomes. Sequences flanking the inverted TTAGGG repeats marking the fusion site are duplicated at multiple, primarily subtelomeric, locations. 150 kbp of sequence on the proximal side of these multi-copy blocks is repeated at 9p24, 9p11, 9q13, and Y (98% average identity); 40 kbp of sequence on the distal side is homologous to sequence at 22qter. We identified 8 genes in this region, all of which are duplicated at least once elsewhere in the genome. Both the forkhead protein 5 (FREAC5)-like and cobalt synthetase W (COBW)-like sequences are present in 5 copies in the human genome (2q13, 9p24, 9p11, 9q13 and Y). By comparing the sequences of these copies and transcripts, we have established that FREAC5-like genes on 2q13 and 9p24 are transcriptionally active; only the copy on the Y is clearly a pseudogene. The 9p24 copy of the COBW-like gene is expressed; the other 4 are potentially functional. 2q13 houses a functional copy of SNRPA1, while the subtelomeric copy on 22 is a pseudogene. In contrast, RABL2 genes on both 2q13 and 22qter are active (Wong et al., 1999). The 2q13 region also includes two pseudogenes (similar to ribosomal protein L23A and a yeast helicase, CHL1) and fragments of genes for phosphoglucomutase related protein and acrosin. We have also identified a large CGG repeat ~1Mb from the fusion site as a possible candidate for the FRA2B fragile site.

Program Nr: 19 from the 2001 ASHG Annual Meeting

Evolution of regulatory elements in primates and mouse: conservation and turnover. *E.T. Dermitzakis, A.G. Clark.* Dept of Biology, Penn State University, University Park, PA.

Sequence comparisons between human and rodents are widely used for the identification of regulatory regions in the human genome (phylogenetic footprinting), and the importance of such inter-genomic comparisons for promoter annotation is expanding. While it is widely appreciated that conservation of sequence motifs may provide a suggestion of function, the pattern of conservation within transcription factor binding sites has not been adequately studied. We present an analysis of the evolutionary dynamics of transcription factor binding sites whose function had been experimentally verified in promoters of more than 40 human genes, and compare their sequence to homologous sequences in other primate species and mouse. Our results suggest that the conservation of background promoter regulatory sequence is similar enough to the embedded binding site sequence that the transcription factor binding sites do not stand out as blocks of highly conserved sequence. In addition, we find that more than 40% of the human functional binding sites are not conserved in mouse. We use binding site prediction and simulations to model the properties of transcription factor binding sites and use the information and methods obtained to quantify the evolutionary parameters of regulatory sequences. We conclude that regulatory sequences have an underlined pattern of evolution not readily observed by sequence comparisons. Our results have a strong bearing on the future of phylogenetic footprinting and the understanding of the evolutionary process in regulatory sequences.

Program Nr: 20 from the 2001 ASHG Annual Meeting

Characterization of L1 insertions in cultured human cells reveals a new role for L1 in genomic rearrangement.
S.M. Lutz-Prigge, N. Gilbert, T. Biagi, J.V. Moran. Human Genetics and Internal Medicine, University of Michigan, Ann Arbor, MI.

L1 comprises ~17% of human genomic DNA, and continues to alter the genome not only by propagating itself, but also by mobilizing sequences derived from its 3' flank (L1-mediated transduction). The L1-encoded proteins also may function *in trans* to mediate Alu retrotransposition and processed pseudogene formation. Moreover, non-allelic recombination between L1s is implicated in genomic rearrangement and disease. L1 integration is most clearly understood by studying new insertion events, but this is hindered by the low frequency of endogenous retrotransposition. We previously tagged L1s with a selectable marker (*mneoI*) that, upon retrotransposition, conferred resistance to G418 ($G418^R$). Here, we describe a system to rapidly characterize new L1 insertions. We modified the *mneoI* cassette to include a dual prokaryotic/eukaryotic promoter and a prokaryotic origin (ColE1) of replication. L1s harboring this cassette readily retrotranspose in HeLa cells, generating $G418^R$ foci. To study newly integrated L1s, genomic DNA was extracted from $G418^R$ cells, digested, circularized, and transformed into *E.coli*. Kanamycin (an analog of G418) resistant *E. coli* contain plasmids with a newly inserted L1 and its flanking sequences. Thus far, analysis of 13 integrants showed the general characteristics of L1 retrotransposition (i.e., 5' truncation, 3' polyadenylation, target site duplication, and preferential insertion at an L1 endonuclease cleavage site). Our data further suggest that internal deletions of L1 can occur during retrotransposition (likely mediated by template switching) and that newly inserted L1s can undergo homeologous gene conversion with endogenous L1s. Gene conversion results in the creation of a chimeric L1, and the apparent concomitant deletion of intervening genomic sequence. Thus, L1 is not only an insertional mutagen, but its retrotransposition may also result in large (> 1kb) deletions of genomic sequence.

* These authors contributed equally to this work.

Program Nr: 21 from the 2001 ASHG Annual Meeting

A novel active L1 retrotransposon subfamily in the mouse substantially increases the number of known active mouse L1s. *J.L. Goodier, E.M. Ostrertag, K. Du, H.H. Kazazian, Jr.* Genetics, University of Pennsylvania, Philadelphia, PA.

Mouse L1 elements have a structure similar to that of human L1s, containing a 5' UTR with promoter activity, 2 ORFs, a 3' UTR, and a poly A tail. One difference is in the 5' UTR, where mouse L1s have tandemly repeated 200 bp regions called monomers. The multiple L1 subfamilies in mouse are distinguished by their monomer sequences. Previously, nearly all active mouse elements belonged to a young L1 subfamily called T_F, which potentially contains 2000 active elements among its 3000 full-length members. Another mouse subfamily, the A subfamily, contains 6-8000 full-length elements. We have now isolated 14 L1s from this subfamily and found that 2 of them (14%) are active. We then searched the mouse genome database for new L1 subfamilies and discovered a novel subfamily, which we named G_F. This subfamily has a unique monomer sequence and unusual patterns of monomer organization. A majority of these G_F elements also have a unique length polymorphism in ORF1. About 1500 full-length G_F elements exist in the diploid mouse genome. Polymorphism analysis of G_F elements in several mouse subspecies and lab strains revealed that, like T_F, the G_F subfamily is young and expanding. Based on a cultured cell retrotransposition assay of 5 cloned G_F elements, about 400 G_F elements in the mouse genome are potentially capable of retrotransposition. These data now increase the number of known active mouse L1 subfamilies from one (T_F) to three (T_F, A, and G_F). In contrast to the roughly 60-90 active L1s in humans, mice have approximately 3100 active L1 elements, 1800 T_F, 900 A, and 400 G_F.

Program Nr: 22 from the 2001 ASHG Annual Meeting

Genetic signatures of migrations and population replacements during human colonization of the North American Arctic. *M.G. Hayes, D.H. O'Rourke.* Anthropology, Univ Utah, Salt Lake City, UT.

The eastern North American Arctic is characterized by a striking prehistoric cultural transition ca. 1000BP when the Thule whaling culture abruptly replaces the existing Dorset culture. Ancient DNA was extracted from Dorset and Thule culturally associated skeletal remains, and four to six polymorphic mtDNA restriction site and length markers characterizing maternal haplogroups were analyzed. The Thule (n=20), like the living Inuit, are fixed for haplogroup A, while the Dorset (n=3) are fixed for haplogroup D. A Fisher‘s exact test (p=0.006) and a principal components analysis (PCA) of these haplogroup frequencies clearly distinguish the two populations, suggesting a genetic replacement accompanied the observed cultural replacement. A third population, the Sadlermiut, a proto-historic group hypothesized to be a remnant Dorset population isolated from the Thule, were also analyzed. The Sadlermiut (n=19) have approximately equal frequencies of haplogroups A and D. The PCA places the Sadlermiut intermediate between the Thule and Dorset, and Fisher‘s exact tests yielded statistically significant differences between the Thule and the Sadlermiut (p=0.003), but not the Dorset and Sadlermiut (p=0.474). This suggests the Sadlermiut may indeed be a remnant Dorset population with subsequent gene flow from the Thule. The Aleutian Islands of the western North American Arctic are also characterized by a striking prehistoric transition ca. 1000BP, although it is morphometrically defined with the Paleo-Aleut population dolichocranic and the later Neo-Aleut population brachycranic. Ancient mtDNA was extracted, and the markers described above were analyzed, from both Paleo- (n=16) and Neo-Aleut (n=17) remains. These populations have similar haplogroup frequencies (28-42% A, 58-71% D) regardless of geographic location, and hence they are statistically indistingushable from one another using a Fisher‘s exact test (p=0.387) and PCA. This suggests that neither migration of a new population, nor subsequent population replacement event, occurred in Aleutian prehistory at this time. mtDNA HVS I sequences from these samples are being examined to elucidate this phenomenon.

Program Nr: 23 from the 2001 ASHG Annual Meeting

Genetic structure of a 2500-year-old human population in China. *L. Wang[1], N. Saitou[2], S. Ueda[3].* 1) Department of Medical and Animal Genetics, Institute of Genetics, Chinese Acdemy of Sciences, Beijing, China; 2) Laboratory of Evolutionary Genetics, National Institute of Genetics, Mishima, Japan; 3) Department of Biological Sciences, Graduate School of Science, University of Tokyo, Tokyo, Japan.

The earliest dynasty of China, Xia, was established 4000 years ago in 2070 BC, and was subsequently followed by the Shang, Zhou, Spring-Autumn, Warring States, Qin, Han eras and so on. We have previously investigated temporal changes in the genetic structure of ancient human populations of China (Oota et al. 1999 and Wang et al. 2000). In our previous study, we used the 185-bp mitochondrial D-loop sequences (positions 16194-16378). We here newly obtained 251-bp sequences by using two sets of new primers that amplify the overlapping mitochondrial D-loop regions (positions 1594616185 and 1596716216). All the samples were collected at Linzi, in the Shandong Province of China. Linzi was the capital of Qi, one of the feudal states in the Spring-Autumn and the Warring States eras, and located near the mouth of the Yellow River in ancient times. We succeeded in PCR amplification for 31 of the 63 2,500-year-old (Spring-Autumn era) remains and 50 of the present-day Han Chinese living in Linzi. The nucleotide sequences of both strands in each DNA region were determined. The 436-bp nucleotide sequences from both of the present and our previous studies were employed for statistical analyses. Phylogenetic tree and mult-dimensional scattergram for the 436-bp sequences were compared with those for the 185-bp sequences in our previous study. Inconsistent with the geographical distribution, the 2500-year-old Linzi population showed greater genetic similarity to the present-day European populations than to the present-day East Asian populations. The genetic backgrounds of the 2500-year-old and the present-day Linzi populations were distinct from each other. These results indicate that the genetic structure of the human population changed drastically during the 2500 years at the lower reaches of the Yellow River.

Program Nr: 24 from the 2001 ASHG Annual Meeting

Y chromosome and mtDNA variation in linguistically diverse peoples of Tanzania: Ancient roots and ancient clicks. *A. Knight[1], P.A. Underhill[2], H.M. Mortensen[1], A.A. Lin[2], D. Louis[1], M. Ruhlen[1], J.L. Mountain[1]*. 1) Department of Anthropological Sciences, Stanford University, Stanford, CA; 2) Department of Genetics, Stanford University, Stanford, CA.

We analyzed genetic variation in 122 "unrelated" individuals from the vicinity of Lake Eyasi in north-central Tanzania, to recover aspects of population history and human evolution. Representatives of the four linguistic phyla of Africa were studied, including 50 Hadzabe (or Hadza) foragers. We present a Y chromosome phylogeny derived from unique event polymorphisms (UEPs). We also present Y chromosome microsatellite variation within UEP-defined clades, mitochondrial DNA (mtDNA) fragment polymorphisms, and nucleotide sequences of both mtDNA control region hypervariable segments. When compared to other African population data, our results reveal elements of prehistory relevant to the evolution of anatomically modern humans, including migration, gene flow, and language. We were able to distinguish recent gene flow from ancient demographic signatures. Hadzabe are strongly differentiated from other groups, have high genetic diversity, and exhibit comparatively great genetic distance from the !Kung of southern Africa, suggesting great antiquity for click (Khoisan) languages.

Program Nr: 25 from the 2001 ASHG Annual Meeting

Out of Africa expansion does not represent a random sampling of Sub-Saharan lineages. *D. Labuda[1, 2], E. Zietkiewicz[1], V. Yotova[1], I. Arrieta[3], M. Batzer[4], D.E.C. Cole[5], J. Jaruzelska[6], D. Modiano[7], J.P. Moisan[8], R. Michalski[9].* 1) Research Center, Hospital Sainte-Justine, and; 2) Pediatrics Dept, U of Montreal, Montreal, QC, Canada; 3) UPV, Bilbao, Spain; 4) LSU, Baton Rouge, LA, USA; 5) U of Toronto, Toronto, ON, Canada; 6) Polish Acad Sci, Poznan, Poland; 7) ULS, Rome, Italy; 8) CHRU, Nantes, France; 9) Victoria Hospital, Prince Albert, SK, Canada.

Dys44 haplotypes consist of 35 polymorphisms from an 8kb segment of the dystrophin gene on Xp22. The analysis of over 1300 chromosomes representing populations from all over the world revealed 74 different haplotypes. They can be divided into 10 families, each grouping haplotypes that are structurally related: 3 of these families are unique to Africa, 7 other are found on different continents. It appears that African chromosomes descend from at least two lineages that evolved separately for a period of time: one of them underwent range expansion colonizing different continents, including Africa where it mixed with the other one (African-specific haplotypes). This scenario explains the general observation of the greater diversity of sub-Saharan populations and at the same time reveals a novel aspect of early human evolution. The analysis of geographic distribution of the 7 worldwide haplotype families makes it possible to retrace the routes of modern humans expansion outside Africa. The most ubiquitous B001 haplotype is found at the highest frequency in Eurasia and Americas. Haplotypes B002 distribution follows the southern route, linking Africa, South-East Asia, Indonesia and Papua New Guinea. In contrast, haplotypes B003 and B006 indicate "northern route" connecting Europe, Asia and Americas. A dramatic increase in B004 frequency in Amerindians from South and Central America and the absence of B005 from all Paleoindian and NaDene populations can be associated with the colonization of the Americas. Our diversity data indicate that the dispersal of the expanding lineage was relatively recent (presumably in the Upper Paleolithic); they also show that the peopling of the World by modern humans occurred through a series of founder effects. (*Supported by CIHR*).

Program Nr: 26 from the 2001 ASHG Annual Meeting

Geographic Distribution of Three Tay-Sachs Mutations in Ashkenazim Supports Drift over Selection. *N.J. Risch[1], H. Tang[1], H. Katzenstein[2], J. Ekstein[2].* 1) Dept Genetics, M322, Stanford Univ Medical Ctr, Stanford, CA; 2) Dor Yeshorim, Brooklyn, NY.

The selection versus drift debate over lysosomal storage diseases in the Ashkenazi Jewish (AJ) population has a long history. Here we provide new data relevant to this topic by studying the European geographic distribution of 3 Tay-Sachs (TSD) mutations found in the AJ population: 1277TATC, +1IVS12, and G269. Over 62,000 subjects were tested for the three mutations as part of the Dor Yeshorim Screening Program. These subjects also reported geographic origins for their four grandparents. Sufficient numbers were available to estimate allele frequencies for the following countries/regions: Hungary, Czechoslovakia, Germany, Austria, Romania (Central Europe), Lithuania, Russia, Poland (Eastern Europe), the Mediterranean, and Middle East.

As had been reported previously from enzyme detected carriers, we detected a higher frequency of the predominant TSD mutation (1277TATC) in Central versus Eastern Europe. However, the other two mutations (+1IVS12 and G269) were found at significantly higher frequency in Eastern Europe than Central Europe. In fact, the +1IVS12 mutation was completely absent in Central Europe, but had a frequency of 1.1% in Lithuania and 0.7% in Russia, consistent with previously identified recently-arisen mutations for torsion dystonia and LDL-recepter deficiency in this population.

The disparate geographic distribution of the three TSD mutations and the likely recent origin of the second most common mutation in Lithuanian Jews argues strongly against the role of selection in creating the observed allele frequency distribution of TSD mutations in the Ashkenazi population.

Program Nr: 27 from the 2001 ASHG Annual Meeting

Human population substructure and its influence on association studies. *D.J. Cutler[1], M.E. Zwick[1], C.T. Yohn[1], K.P. Tobin[1], C. Kashuk[1], N.A. Shah[2], J.A. Warrington[2], A. Chakravarti[1]*. 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine; 2) Affymetrix Inc., Santa Clara, CA.

Genome wide association studies within a case-control experimental design have two principle advantages over family-based linkage analysis: study samples are easier to collect, and for the same number of samples, there is greater power. Association studies also suffer from one principle disadvantage. Population substructure, or stratification, can create false associations between disease and genetically unlinked regions. Although stratification certainly might create difficulties, whether or not there actually is enough substructure among human populations to interfere with association studies is strictly an empirical question. The strength of this effect was examined in a set of 40 cosmopolitan U.S. samples whose ancestors originated on four continents. Approximately 50kb of unique DNA was resequenced using high-density oligonucleotide arrays fom 40 loci, generating nearly 2 megabases of sequence for each individual. Population stratification in this data set was assessed in three ways, by its effect on: 1) the allele frequency spectrum, 2) genotype frequencies, 3) association between genetically unlinked genomic regions. All three approaches gave consistent answers. There is substructure to the human population, but its effects are weak. FST, a measure of substructure, is 7.1% averaged across all loci, with a weak trend towards higher frequency sites showing more subdivision. Overall this value is consistent with modest differences in allele frequencies between populations. With p=10-3, a case-control study conducted with this highly stratified set of samples is likely to find a false positive association to an unlinked genetic region only 1-2%; of the time. Moreover, the proportion of correlated sites is consistent across loci. Thus, we believe that a genome wide, case-control association study, with proper correction for multiple tests and correlation across loci, is the best first stage to any mapping study. Transmission based testing in a second sample can rule out rare false positives.

Program Nr: 28 from the 2001 ASHG Annual Meeting

Fine-structure haplotype map of 5q31: implications for gene-based association studies and genomic LD mapping.
M.J. Daly, J.D. Rioux, S.F. Schaffner, T.J. Hudson, E.S. Lander. Whitehead Institute, Cambridge, MA.

Linkage disequilibrium (LD) mapping is theoretically a powerful tool for disease gene localization but has been difficult to deploy in practice. Primarily this has been due to the fact that the power of any particular marker to detect association due to LD with a causal mutation is strongly dependent not only on distance but also on the relative history and frequency of the marker and the mutation. A comprehensive haplotype-based analysis of LD offers great advantages for gene mapping but depends on the actual underlying structure of LD and the development of appropriate analytic tools. In the context of our positional cloning efforts in Crohn's disease we performed a detailed study of variation patterns across 500 kb on 5q31. In this study, we observed strikingly limited haplotype diversity across long distances punctuated by sites of multiple historical recombination events. Essentially, this long genomic region can be parsed into blocks of low diversity in which recombination plays little or no role in assortment of haplotypes and between which recombination has been much more active in creating new haplotypes, suggesting the presence of recombinational hotspots. The description of these haplotype patterns suggest powerful methods for testing for association. Specifically, the non-recombining regions with limited haplotype diversity can be treated as single, multi-allelic loci that represent the true manner in which individuals differ from one another genetically and as such, are the quantum of genetic variation that should be tested for association. Furthermore, active sites of recombination provide precise boundaries across which association to phenotype will change considerably. This has led us to develop a Hidden Markov Model for the interpretation and testing of haplotype data in this fashion through the assignment of observed chromosomes to common haplotype patterns and identification of sites of likely historical recombination. The haplotype map described here provides a prototype for a genome-wide variation map.

Program Nr: 29 from the 2001 ASHG Annual Meeting

HypSNPs and hypHaps: unstable haplotypes in the human genome. *C. Ouyang[1], L. Geller[1], J-E. Gustaveson[1], M. Beaulieu[1], G.P. Larson[1], S.D. Flanagan[2], P. Rodley[1], B. Shannon[1], C. Deckman[1], C.G. Lundberg[1], T.G. Krontiris[1]*. 1) Division of Molecular Medicine, Beckman Research Institute of the City of Hope National Medical Center, Duarte, CA; 2) Division of Neuroscience, Beckman Research Institute of the City of Hope National Medical Center, Duarte, CA.

We analyzed eight short PCR-amplified regions (average size ~600 bp) evenly scattered over chromosome 22. Each region contained a known single nucleotide polymorphism (SNP) with a minor allele frequency greater than 0.2, based on information available from public databases. DNA sequencing of PCR products from 22 individuals showed that seven of these eight regions contained multiple additional SNPs, also with relatively high allele frequencies. Four of the eight regions unexpectedly demonstrated multiple haplotypes, despite the short physical/genetic distances involved. There was no correlation of the degree of haplotypic variation at any given locus with known regions of chromosome 22 bearing higher recombination rates. Detailed analyses revealed that haplotypic variation was the result of specific, hypervariable SNPs (hypSNPs) that were most often distributed on only one of the two most common haplotypes at each locus. HypSNPs resided as close as 2 bp away from stationary SNPs defining the two common haplotypes. Examination of a larger region (28 kb) surrounding the *CDKN1A* gene on chromosome 6 also revealed the presence of hypSNPs; again, one particular haplotype demonstrated the most variation. Sites of variation were associated with multiple crossover events. We discuss the implications of these results for human genetic variation and for disease gene association studies.

Program Nr: 30 from the 2001 ASHG Annual Meeting

When is haplotype analysis advantageous for linkage-disequilibrium mapping? *R.W. Morris, N.L. Kaplan.*
Biostatistics Branch, NIEHS, RTP, NC.

Recent proposals for using haplotype analyses, in contrast to single-site analyses, to identify and locate disease-predisposing genes raise a question of when analyses based on haplotypes will be more efficient than analyses based on single markers. We hypothesize that haplotype analysis dominates single marker analysis when there is allelic heterogeneity at a complex disease-predisposing locus. To investigate this hypothesis, we employ a permutation framework to study properties of a haplotype-based strategy for fine-mapping a multiallelic susceptibility gene using preferential transmission of haplotypes in nuclear families. We focus on a one-sided alternative, because in the presence of linkage and linkage disequilibrium a subset of marker haplotypes should be over-represented in gametes transmitted to affected offspring and under represented in gametes not transmitted to affected offspring. To study the properties of alternative analysis strategies we use a coalescent model to simulate haplotype data assuming tight linkage. Using these ideal data, we compare the results of haplotype and single-site analyses that attempt to locate a disease-predisposing gene with multiple disease-predisposing alleles. We also investigate the consequences for haplotype analysis of varying the number of contiguous sites included in a haplotype as well as the fraction of phenocopies among affected offspring. Finally, we investigate the loss of performance anticipated in a more realistic scenario when using multiple marker genotype data in which haplotypes are not known and must be reconstructed.

Program Nr: 31 from the 2001 ASHG Annual Meeting

Complex SNP haplotypes at ATM and BRCA1 show potential association of ATM and BRCA1 variants with sporadically occurring breast cancer in Ashkenazi Jewish women. *P.E. Bonnen[1], A. Figer[3], S. Arbel[2], R. Bruchim[2], E. Friedman[2], D. Nelson[1]*. 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Chaim Sheba Medical Center, Tel-Hashomer, Israel; 3) Tel-Aviv Medical Center, Tel-Aviv, Israel.

We hypothesize that commonly occuring variants of genes involved in maintenance of DNA integrity can represent low penetrance alleles that influence cancer risk. Our strategy for detecting these risk-confering alleles is to develop complex SNP haplotypes for genomic regions (130 -180kb) containing candidate cancer genes and to perform association studies in cancer populations. We used a resequencing approach to discover SNPs (8-14) spanning the length of each locus of interest (ATM, BRCA1, and BRCA2). We then genotyped an ethnically defined control population (~50 Afam, ~50 Asian, ~50 Hisp, ~50 Cauc) 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 and BRCA1 having 22 and 15 haplotypes respectively while BRCA2 had 47. Examination of linkage disequilibrium (LD) and recombination across these loci supported the notion of locus-specific genomic diversity with ATM and BRCA1 showing strong LD and BRCA2 extensive recombination. Currently underway is an association study in Ashkenazi Jewish with sporadic breast cancer prescreened for and found not to be carriers of the three common BRCA1 & BRCA2 mutations, and ethnically and age matched controls. Preliminary results with ~100 cases and ~100 controls reveals a trend for two ATM and two BRCA1 haplotypes. In each gene one haplotype has increased frequency in cases and another has increased frequency in controls, thus one haplotype may lend risk and the other a protective effect. Interestingly these four associating haplotypes are high frequency in all study populations (0.38-0.11) and as such may represent commonly occurring variants influencing risk for cancer. Additional samples are being typed for these genes as well as BRCA2, RAD51 and TP53 with the ultimate goal of examining complex interactions among variants of these genes.

Program Nr: 32 from the 2001 ASHG Annual Meeting

A comparison of single nucleotide polymorphism(SNP) and microsatellite genome scans for May-Hegglin anomaly(MHA). *E.W. Pugh[1], P.D. Witmer[1], J.W. Wanyee[1], Y.T. Fan[1], G.P. Ibay[1], A. Voltz[2], E. Luong[2], J.L. Goldstein[1], R.L. Nussbaum[2], J.F. Korczak[3], M.J. Kelley[4], K.D. Doheny[1].* 1) CIDR, JHU, Baltimore, MD; 2) IDRB,NHGRI,NIH, Bethesda, MD; 3) Karmanos Cancer Inst, Detroit, MI; 4) Duke University, Durham, NC.

To assess the utility of SNPs for linkage analysis, we compared genome scan linkage results for MHA using microsatellite markers and SNPs in three families. MHA was linked to chromosome 22q12-13 (Hum Genet 106:557-564) using microsatellite markers genotyped at the Center for Inherited Disease Research and the mutant gene (MHY9) identified (Nature Genet 26:106-108). We genotyped the same families with 1,494 SNP markers using the GeneChip® HuSNP™ Mapping Assay and Microarray Suite 4.0 software (Affymetrix). Chromosome 22 markers were ordered using finished sequence. Consensus locations (Affymetrix) were used for other chromosomes. Of the 1,462 autosomal SNP markers, 1016 (70%) had max lod scores >.05 or < -.05, 96 (7%) had little information for linkage and 350 (24%) failed. The average error rate for the Affymetrix reference sample and for 5 experimental samples run in duplicate was 0.32%(+/- 0.11%, median 0.29%) and 1.55%(+/- 2.19%, median 0.64%), respectively. Two point lod scores were calculated using FASTLINK for the SNP markers using the same disease model as the original analysis. The highest max lod scores flank the gene (MYH9,15.9Mb) on contig NT_001454 at WIAF-468(3.7Mb, lod 3.06,q=0.001) and WIAF-3918(20.8Mb, lod 2.75,q =0.001). The maximum two point lod in the microsatellite genome scan was 3.91, q=0.076, at D22S683 (36cM, 15.7Mb). Highly negative lod scores were found on chromosomes other than 22 (626 SNP's lod < -2.0, q=0). No max lod above 1.5 was found on other chromosomes, comparable to the original genome scan. Although the max lod scores were lower and there was a large proportion of missing or uninformative data, we were able to replicate linkage of this single gene disorder to chromosome 22 using a SNP genome scan. We are genotyping additional SNPs on chromosomes 21 and 22 using the Homogeneous MassEXTEND (hME) assay (Sequenom) to investigate optimal spacing and number of SNPs for linkage analysis.

Program Nr: 33 from the 2001 ASHG Annual Meeting

Methods for analysis and visualization of SNP genotype data for complex diseases. *A. Tsalenko[1], A. Ben-Dor[1], Z. Yakhini[1], N.J. Cox[2]*. 1) Agilent Laboratories, Palo Alto, CA; 2) University of Chicago, Chicago, IL.

The emergence of SNP markers as a central method for studying genetic determinants of complex diseases and the amount of SNP data collected in such studies call for the development of specialized analysis tools. We present novel algorithmic and statistical approaches to ranking SNPs using different scoring schemes; visualizing and interacting with SNP data; assessing joint contributions of multiple SNPs and finding small subsets of SNPs that could accurately predict diagnosis status or other meaningful properties. Our methods are implemented in a new Matlab based prototype package "SNPTool" developed on the basis of tools used for analysis of gene expression data (J Comp Bio 2000; 7559-83, Nature, Aug 2000; 406: 536-40). We analyzed 120 SNPs typed in Mexican-Americans with type II diabetes (Nat Genet, Oct 2000; 26: 163-75), 89 SNPs were on chromosome 2 in the NIDDM1 region, and 31 SNPs were on chromosome 15 in the CYP19 region. One patient from each of 108 affected sib-pairs was selected into a set of representatives. These patients were partitioned into 3 groups: patients from families that showed evidence for linkage in the NIDDM1 region (38), patients from families with evidence against linkage (16) and patients from families with linkage signal close to 0 (54). For each SNP, we computed the mutual information of the corresponding genotypes with this partition into three sets, and the corresponding significance level (p-value). Mutual information measures the amount of information the genotype at each SNP gives about membership in each one of these three sets. We identified 10 most informative SNPs with p-values less than 0.05. Interestingly, 7 of these are tightly linked polymorphisms in the calpain-10 gene; others are linked SNPs from the region between GPR35 and ATSV genes. Using backward sequential search method we identified a subset of 11 SNPs from chromosome 2 such that their combined genotypes predicted the 'linkage status' with 87% accuracy. Similarly, a set of 11 SNPs from chromosomes 2 and 15 was found that predicted the 'linkage status' with 90% accuracy. Supported by DK-55889, DK-47486, DK-20595 and Agilent Laboratories.

Program Nr: 34 from the 2001 ASHG Annual Meeting

Linkage Disequilibrium and Genetic Diversity in the ADH Genes. *M.V. Osier[1], A.J. Pakstis[1], J.R. Kidd[1], B. Bonne-Tamir[2], R.-B. Lu[3], H. Soodyall[4], K.K. Kidd[1]*. 1) Hum Genet,Yale,New Haven,CT; 2) Tel-Aviv U,Tel-Aviv,Israel; 3) Nat Def Med Ctr,Taipei,Taiwan; 4) SAIMR,WITS U,Johannesburg,S Afr.

Variants of the Class I alcohol dehydrogenase genes have been shown in several studies to be associated with a protective effect against alcoholism. Previous work from our lab has shown that linkage disequilibrium (LD) is a confounding factor in such studies (Osier et al., 1999, AJHG 64:1147-1157). Here we describe an initial study into the nature of variation in population samples from different regions of the world and the extent of LD in the ADH gene family as a whole (ADH1B, ADH1C, ADH4, and ADH7). We have determined allele frequencies in 35 population samples from around the world for five previously described sites (ADH1B Exon 3 coding, RsaI, Exon 9 coding; ADH1C Exons 6 and 8 coding) plus four new sites (ADH1C EcoRI, HaeIII; ADH7 StyI; ADH4 Hpy188I). For the nine-site haplotypes, expected heterozygosities are greater than 0.54 for all populations studied, even though estimated heterozygosities for the four individual ADH1C sites are often less than 0.10 in East Asian populations and the ADH1B Exon 9 site is only observed as polymorphic in Africa. Significant pairwise LD (measured as D', $p<0.05$, $1.00>|D'|>0.273$) was observed between sites as far away as the ADH7 StyI and ADH4 Hpy188I coding variant (~296kb) in eight populations (Biaka, Yoruba, Ethiopians, Yemenites, Druze, Adygei, Danes, MX Pima). In 4 of the 7 African populations in which both alleles were observed at the ADH7 StyI and ADH1B Exon 9 coding variant sites (~111kb), significant pairwise LD ($p<0.01$, $1.00>|D'|>0.543$) was observed. Strong ($8.65>x>1.15$) and highly significant ($p<0.001$) overall LD was observed across all seven Class I ADH sites (ADH1B,ADH1C) except in the Ami and Atayal ($x>0.58$, $p<0.002$). LD was nearly as strong across the region with the seven Class I sites and the ADH4 Hpy188I site in all populations studied ($x>0.43$, $p<0.004$, 224kb). The strong LD extending across most, usually all, members of this gene family implies that LD must be considered a likely confounding factor in studies designed to identify the variants truly relevant to protection against alcoholism. (NIAAA grant AA09379 to KKK).

Program Nr: 35 from the 2001 ASHG Annual Meeting

Association of prion protein gene single nucleotide polymorphism haplotypes with Creutzfeldt-Jakob disease.

S.H. Mead[1], S.P. Mahal[1], M. Farrall[2], E.M.C. Fisher[1], J. Collinge[1]. 1) The MRC Prion Unit, Imperial College St Marys Hosp, London, England; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Headington, Oxford OX3 7BN, UK.

The UK population has been widely exposed to bovine spongiform encephalopathy prions, the aetiological agent of variant Creutzfeldt-Jakob disease. Epidemiological experience of this and other episodes of human population exposure to prions show that both prion disease susceptibility and incubation time are highly variable. The host prion protein gene contains a strong genetic susceptibility factor for all forms of prion disease, the codon 129 genotype, which in variant CJD patients to date is universally methionine homozygous. We wish to test the hypothesis that a component of human prion disease susceptibility is determined by factors tightly linked to the prion protein gene. Eight CEPH parents were sequenced over 35kb of the prion protein gene locus in order to identify nearby common single nucleotide polymorphisms. 58 polymorphic sites were characterised including coding changes in the prion protein-like doppel gene. Twelve of these SNPs were genotyped by restriction digest in the 61 CEPH families to define eight European prion protein gene haplotypes. 434 haplotypes comprised of these twelve SNPs were then estimated in a collection of prion disease samples of different aetiologies. A significant association was found between haplotype and sporadic CJD. There was no association however, between haplotype and early cases of variant CJD. In addition, there was no evidence of a prion protein gene founder effect in a known geographical cluster of variant CJD.

Program Nr: 36 from the 2001 ASHG Annual Meeting

Contrasting Genetic Influence of Alleles in the MCP-1 - MCP-3 - EOTAXIN Chemokine Cluster on HIV-1 Transmission and AIDS Progression. *W.S. Modi[1], C. Winkler[1], J.J. Goedert2[2], D. Vlahov[3], S. Buchbinder[4], J. Phair[5], S. Donfield[6], S.J. O'Brien[7].* 1) SAIC, NCI-Frederick Cancer Res, Frederick, MD 21702; 2) Viral Epidemiology Branch, National Cancer Institute, Rockville, MD 20852, USA; 3) Johns Hopkins School of Hygiene and Public Health for AIDS Link to the Intravenous Experience, Baltimore, MD 21205, USA; 4) San Francisco Department of Public Health, San Francisco, CA 94102; 5) Department of Medicine, Northwestern University Medical School, Evanston, IL 60611; 6) Rho, Inc., Chapel Hill, NC 27514, for Hemophilia Growth and Development Study; 7) Laboratory of Genomic Diversity, National Cancer Institute-FCRDC, Frederick, MD 21702-1201.

Nine single nucleotide polymorphisms (SNPs) in a 35 kb region encompassing these genes have been genotyped in 2300 patients from five USA based natural history AIDS cohorts. Ninety-five percent of the variation is explained by five haplotypes in Caucasians and seven in African Americans, indicating strong disequilibrium and similar frequencies among several of the variants. One haplotype contains three SNPs, occurs at a frequency of 20% in Caucasians, extends 31 kb, and includes all three genes. This haplotype is present at a significantly elevated frequency in highly exposed yet persistently uninfected subjects compared to HIV-1 positive individuals. A putative mutation modifying the last amino acid of the eotaxin signal peptide is associated with increased susceptibility to infection among Caucasian hemophiliacs. Two SNPs, which reside on different haplotypes, revealed contrasting effects on disease progression among African Americans. One polymorphism in the promoter region of eotaxin is significantly associated with an increased rate of progression to AIDS and death. Alternatively, a substitution in exon 2 of MCP-1 is associated with a retarded rate of progression to AIDS. The ligands from each of these genes use receptors (CCR2 and CCR3) that are also utilized by HIV-1 to infect cells. In addition, MCP-3 and eotaxin are known to stimulate anti-HIIV-1 cytotoxic T-cells. The complex disease associations discovered may reflect heterogeneity in gene regulation among the different genetic backgrounds.

Program Nr: 37 from the 2001 ASHG Annual Meeting

Further delineation of cardiac abnormalities in Costello syndrome. *A. Lin[1], P. Grossfeld[2], R. Hamilton[3], L. Smoot[4], K. Gripp[5], V. Proud[6], R. Weksberg[3], P. Wheeler[7], J. Picker[4], M. Irons[4], E. Zackai[5], C. Scott, Jr[8], L. Nicholson[8].* 1) Teratology Program, Brigham & Women's Hosp, Boston, MA; 2) UCSD, CA; 3) Hosp. Sick Children, Toronto, Canada; 4) Children's Hospital, Boston, MA; 5) Children's Hospital of Phila., PA; 6) Hosp. King's Daughters, Norfolk, VA; 7) New England Medical Center, Boston, MA; 8) Dupont Hosp. for Children, Wilmington, DE.

We studied 94 pts (27 new, 67 literature) with typical features of Costello syndrome (CS) to better define the type and frequency of cardiac abnormalities. Ages ranged from 2 mos - 32 yrs (23% were less than 2 yrs). A cardiac abnormality was present in 58/94 (62%). A CARDIOVASCULAR MALFORMATION (CVM)was noted in 28 (30%), almost half of whom had pulmonic stenosis. CARDIAC HYPERTROPHY was found in 31 (33%), half of whom had hypertrophic cardiomyopathy of the left ventricle (HCM); right and biventricular involvement also occurred. A RHYTHM DISTURBANCE occurred in 29 (31%), half had atrial tachycardia, often chaotic. The following combinations were noted: CVM + HCM (5), HCM + rhythm (11), CVM + rhythm (4), CVM + HCM + rhythm (5). Conclusions: 1. Since over half of CS pts have a cardiac abn., prospective studies (echo, Holter) should be done on all pts. Cardiac hypertrophy and tachycardia contribute to morbidity and mortality, and are theraeputic challenges. 2. The lack of complex CVMs supports the notion that CS is a dysplasia, not a multiple malformation syndrome. 3. Cardiac hypertrophy is common, often as typical HCM, but inadequately defined. How cardiac "overgrowth" relates to the neonatal macrosomia is unknown. 4. Atrial tachycardia (described as chaotic atrial rhythm, multifocal or ectopic atrial tachycardia) can occur without underlying CVM or hypertrophy, perhaps reflecting an intrinsic conduction disturbance. 5. Although there is no single pathognomonic cardiac abnormality, the constellation of neonatal cardiac hypertrophy, malignant atrial tachycardia, with a hemodynamically and anatomically mild CVM comprise a cardiac "signature" for CS. This should assist in distinguishing CS from syndromes with similar features, eg. Noonan syndrome, CFC syndrome, MPS.

Program Nr: 38 from the 2001 ASHG Annual Meeting

Identification of novel loci influencing highly heritable serum Lipoprotein (a) levels in Caucasian families with myocardial infarction. *U. Broeckel[1], C. Hengstenberg[2], B. Mayer[2], S. Holmer[2], L.J. Martin[3], A.G. Comuzzie[3], J. Blangero[3], G.A.J. Riegger[2], H.J. Jacob[1], H. Schunkert[2]*. 1) Human and Molecular Genetic Center, Medical College of Wisconsin, Milwaukee, WI; 2) Department of Medicine 2, University of Regensburg, Regensburg, Germany; 3) Department of Genetics, Southwest Foundation of Biomedical Research, San Antonio, TX.

Highly heritable plasma levels of lipoprotein (a) [Lp(a)] are associated with an increased risk of atherosclerosis, myocardial infarction, and stroke. While a significant portion of the genetic component is attributed to sequence variations in the apolipoprotein A (*APOA*) gene, this locus does not explain totally the inherited component. The aim of our study was to identify additional loci contributing to Lp(a) levels.

We performed a genome scan in 1,370 individuals in 513 families with myocardial infarction using 376 autosomal markers. Linkage analysis was performed using a variance component approach (SOLAR) with log-transformed values to account for non-normality. A polygenic model incorporating age, height, and weight as significant covariates determined an overall heritability of 97%. We detected significant linkage at the previously described *APOA* locus on chromosome 6q27 with a LOD of 26.99, explaining 73% of the overall heritability. We also detected suggestive linkage signals on chromosomes 1, 3, 4, and 13 (LOD > 2). In an oligogenic analysis, after adjusting for the variance of the *APOA* locus, we identified a novel locus on chromosome 1 with a LOD score of 3.8 and an attributable heritability of 16%. Interestingly, the apolipopotein A2 gene is located at the peak LOD score. In addition, the loci on chromosomes 4 and 5 maintained a suggestive signal (1.9 < LOD > 3.0).

In conclusion, we identified two novel loci contributing to Lp(a) levels in Caucasians. While the *APOA* locus accounts for the majority of the genetic variance, both loci appear to have a measurable effect on the phenotype. Since significant differences in heritability have been described, further studies in different ethnic populations are needed.

Program Nr: 39 from the 2001 ASHG Annual Meeting

Evidence for interaction between nucleotides within the *ApoE* gene. *S.C. Hamon[1], A.G. Clark[2], K.M. Weiss[2], D.A. Nickerson[3], E. Boerwinkle[4], J.H. Stengård[5], C.F. Sing[1]*. 1) Univ of MI, Ann Arbor, MI; 2) Penn State Univ, Univ Park, PA; 3) Univ of WA, Seattle, WA; 4) Univ of TX Health Science Center, Houston, TX; 5) National Public Health Institute, Helsinki, Finland.

Given the ubiquity of protein interactions, it is expected that gene-gene interactions will influence phenotype variation at every level of organismal organization. Clear examples of such epistatic effects are rare in human studies because most population-based gene-phenotype studies in humans measure few variable sites in each gene, assume independence and additivity of nucleotide effects and have much less power to detect interactions than main effects. In this study we measured a large number of variable nucleotides within the gene coding apolipoprotein E (*ApoE*) and tested the assumption of additivity of their effects on interindividual variation in plasma ApoE levels. To accomplish this we applied an over-parameterized general linear model to a sample of 2287 individuals from Jackson MS (16 SNPs), North Karelia, Finland (14 SNPs), and Rochester, MN (13 SNPs). Of the 387 possible tests, 26 pairs of SNPs showed significant evidence (p£0.05) of interaction effects when predicting values of ln plasma ApoE. Of those pairs that showed evidence of a significant interaction: 3 pairs involved SNPs that had no significant marginal (single site) effects, 17 pairs included one SNP that had a significant marginal effect and 6 pairs were composed of 2 SNPs that each had a significant marginal effect. Linkage disequilibrium and physical distance were not predictive of significant non-additivity between pairs of SNPs. Statistical significance of the interaction effects varied among contexts defined by gender and population. Non-additivity between an exonic SNP (bp 3937) and a 3' SNP (bp 4951) was significant in all 3 samples of males. These results illustrate the complexity of the biological relationships that can exist between single SNP variations and quantitative trait variation and document the need for multi-locus statistical models that are consistent with the biological reality that causation involves more than the effects captured by individual SNPs.
Supported by NHLBI grants HL39107 and HL58238-40.

Program Nr: 40 from the 2001 ASHG Annual Meeting

A Mutation in the Cardiac Pacemaker Channel Gene *hHCN4* is linked to Human Sinus Node Disease. *E. Schulze-Bahr[1], D. Isbrandt[2], A. Neu[2], P. Friederich[2], B. Kaupp[3], O. Pongs[2], G. Breithardt[1]*. 1) Molecular Cardiology, Inst. for Arteriosclerosis Res, Muenster, Germany; 2) Institute for Neural Signal transduction, ZMNH, Hamburg, Germany; 3) Inst. fuer Biologische Informationsverarbeitung, Forschungszentrum Juelich, Germany.

Cardiac pacemaker activity in the human sinus node depends on a slow phase of membrane depolarization between action potentials which is mediated by a variety of ion currents. Among these, the hyperpolarization-activated cationic current I(f) is a key component for the control of rhythmic pacemaking activity and is characterized by a sensitivity due to changes in intracellular cAMP concentrations. The two kinetic components of I(f) are mediated by proteins encoded by the hHCN4 (slow component) and hHCN2 gene (fast component). Here, we performed a candidate gene approach in patients with idiopathic SND in hHCN4. First, the genomic structure has been delineated (8 exons) and the gene was localized to chromosome 15q23-q24.1 using the Genebridge 4 radiation hybrid panel. Direct sequencing of the complete hHCN4 gene revealed a heterozygous missense mutation (del1631C) in exon 5 that is predicted to cause a truncated channel protein (573X) lacking also the cyclic nucleotide binding domain (cNBD) at the C-terminus. Expression studies in transfected COS-7 cells showed that the mutant protein was regularily transported to the cell membrane and mediated an I(f) current with activation properties comparable to wildtype currents. However, increasing cAMP concentrations failed to enlarge I(f) amplitude, a key feature during sympathetic stimulation to accelerate the heart rate. Therefore, absence of the cNBD due to the identified mutation provides a likely explanation for the observed clinical phenotype (sinusbradycardia and chronotopic incompetence). We show for the first time a molecular basis for 'idiopathic' SND in humans, a novel cardiac ion channel disorder that is caused by dysfunction of a cardiac pacemaker channel.

Program Nr: 41 from the 2001 ASHG Annual Meeting

Truncating mutations in the *glomulin* gene cause glomuvenous malformations. *M. Vikkula[1], P. Brouillard[1], J.B. Mulliken[2], O. Enjolras[3], M.L. Warman[4], O.T. Tan[5], B.R. Olsen[6], L.M. Boon[1,7]*. 1) Lab. of Human Molec. Genetics, Christian de Duve Institute and Universite catholique de Louvain, Brussels, Belgium; 2) Vascular Anomalies Center, Childrens Hospital, Harvard Medical School, Boston, MA; 3) Consultation des Angiomes, Hopital Lariboisiere, Paris, France; 4) Department of Genetics, Case Western Reserve University School of Medicine, Cleveland, OH; 5) Tufts University School of Veterinary Medicine, Boston, MA; 6) Department of Cell Biology, Harvard Medical School, Boston, MA; 7) Center for Vascular Anomalies, Universite catholique de Louvain, Brussels, Belgium.

Venous malformations (VMs), localized defects of vascular morphogenesis, are single or multiple bluish-purple lesions that occur mainly in skin and mucosa. They can be dominantly inherited and are among the most frequent lesions in centers that specialize in treatment of vascular anomalies (Vikkula et al., 1998). We previously identified the endothelial-specific angiopoietin receptor TIE2/TEK, located on 9p21, as the cause of mucocutaneous venous malformations (VMCM, MIM 600195) (Vikkula et al., 1996). We have also shown that glomuVMs (MIM 138000), cutaneous venous malformations with smooth muscle-like glomus cells, are linked to 1p21-22 (Boon et al., 1994). In this 4-6 cM *VMGLOM* locus, we identified linkage disequilibrium and narrowed the region to 1.48 Mbp (Irrthum et al., 2001). Herein, we report on the identification of the mutated gene, *glomulin*, localized on the basis of our YAC and PAC maps (Brouillard et al., 2000). We report the complete cDNA sequence, the genomic structure of the gene, and 14 different mutations identified in 20 families. As all but one of the mutations cause premature stop codons, glomuVMs are likely to be caused by loss-of-function of glomulin. These data suggest that glomulin is important for the differentiation of vascular smooth muscle cells, and thus for vasculogenesis and angiogenesis. (see also abstracts: Brouillard et al. and Boon et al.)(vikkula@bchm.ucl.ac.be).

Program Nr: 42 from the 2001 ASHG Annual Meeting

Defects in both type I and II receptor members of the TGF beta superfamily cause inherited plexigenic pulmonary hypertension. *R.C. Trembath[1], J. Thomsom[1], R. Machado[1], N. Morrell[2]*. 1) Division of Medical Genetics, University of Leicester, Leicester, England; 2) Department of Respiratory Medicine, University of Cambridge, Cambridge, England.

Little is known of the pathways critical for the maintenance of the pulmonary vasculature. Pulmonary hypertension, defined as as sustained elevation of pulmonary artery pressure at rest and/or excercise, is associated with a number of coincident disease states, but also as an idiopathic feature of familial primary pulmonary hypension (PPH). We have recently identified heterozygous defects of the type II bone morphogenetic receptor, to underly the majority of cases of familial PPH and many apparently sporadic patients. The majority of these heterozygous mutations predict premature protein truncation supporting haplo-insufficiency as the likely inherited mechanism for disease predisposition. The lack of phenotype (as assessed by gender and age of onset) and genotype correlation likely suggests an important role for additional environmental and genetic factors in the aetiology of PPH.

We have now identified novel heterogeneous germline mutations of the type I receptor termed ALK 1, in 4 multiplex kindreds presenting with unexplained pulmonary hypertension associated with plexigenic pulmonary vascular lesions identical to those seen in familial PPH. Functional analysis, including protein localisation studies by immuno-histochemical analysis, demonstrate high levels of cell specific and maintained expression with normal and diseased pulmonary arteries. Taken together with the known contribution of ALK1 gene defects to the vascular disorder, hereditary hemorrhagic telangectasia, provide strong genetic evidience for a critical role of the endothelial cell in PPH pathogenesis.

Program Nr: 43 from the 2001 ASHG Annual Meeting

TBX5 Mutations Associated with Extensive Phenotypic Heterogeneity in Holt-Oram Syndrome. *Q. Wang[1], M.A. Duhagon[1,2], R. Canessa[2], S. Chen[1], C. Oberti[2].* 1) Center for Molecular Genetics, Department of Molecular Cardiology, and Department of Cardiology, Cleveland Clinic Foundation, Cleveland, OH; 2) Instituto de Cardiologia, Hospital Italiano, Montevideo, Uruguay.

Holt-Oram syndrome (HOS) is an autosomal dominant disorder characterized by congenital heart defects and skeletal malformations. Mutation analysis of TBX5, a gene encoding a member of the T-box family of transcription factors, was performed for a large family and a sporadic patient affected with HOS. A novel 9 amino acid deletion (K126-R134) in the T-box domain of TBX5 co-segregates with 25 patients in the large family and a novel nonsense mutation, W64X, was identified in the sporadic patient. W64X truncates the TBX5 protein by 88%;, leading to the formation of a non-functional protein. The mutation findings were correlated with clinical features. Extensive phenotypic heterogeneity exists in 25 patients carrying the 9-amino acid deletion. The 9-amino acid deletion can cause either cardiac defects (8/25 patients), or skeletal malformations (725), or both cardiac and skeletal defects (10/25). The cardiac defects in the family span a wide spectrum and range from asymptomatic mitral valve prolapse (or/and incompetence), to severe cardiac septation defects, and cardiac death. The cardiac manifestation of disease in the family showed progressively more severe expression in more recent generations, consistent with the possibility that anticipation is involved in pathogenesis of HOS cardiac defects. In conclusion, genotype-phenotype correlation studies established that the TBX5 deletion can cause either cardiac defects or skeletal defects, or both, and uncovered possible involvement of anticipation in development of HOS cardiac defects. The location and character of the mutations identified in this study expand the spectrum of TBX5 mutations, and support a model of haploinsufficiency as the molecular mechanism for Holt-Oram syndrome.

Program Nr: 44 from the 2001 ASHG Annual Meeting

BMP signaling is required for septation of the outflow tract of the heart. *E.C. Delot[1,2,3], M.E. Bahamonde[1,4], M. Zhao[1], K.M. Lyons[1,4,5]*. 1) Departments of Orthopaedic Surgery; 2) Human Genetics; 3) Pediatrics; 4) Biological Chemistry; 5) MCDB; UCLA, Los Angeles, CA.

Bone Morphogenetic Proteins (BMPs) constitute a family of ~20 growth factors involved in a tremendous variety of embryonic inductive processes. In humans, mutation in the BMP receptor BmprII, thought to lead to haploinsufficiency, is the main cause for Primary Pulmonary Hypertension (PPH), suggesting that organogenesis may be sensitive to variable levels of BMP signaling. To test this hypothesis, we engineered a knockout mouse that expresses a hypomorphic form of BMPRII, which lacks half of the extracellular ligand-binding domain, BMPRII^{DE2}.

Mice homozygous for this modified receptor exhibit cardiovascular defects (different from those seen in PPH), demonstrating that the development of vascular tissues is exquisitely sensitive to levels of BMP signaling. The most striking phenotype occurs in the outflow tract of the heart, with absence of septation of the conotruncus below the valve level and interrupted aortic arch, a phenotype known in humans as Persistent Truncus Arteriosus (type A4). Marker analysis and histology demonstrate that the epithelial-to-mesenchymal transformation responsible for the formation of the outflow tract septum is initiated but not sustained in mutants, and that the defect is not due to a global defect in neural crest migration.

In addition, semilunar valves do not form in mutants, while the atrioventricular valves appear unaffected. This result challenges the widely held view that semilunar and atrioventricular valves form through a common mechanism. Abnormal septation of the heart and valve anomalies are the most frequent forms of congenital cardiac defects in humans; however the embryologic processes that lead to the formation of these tissues are very poorly understood, and most mouse models display broad defects throughout cardiac tissues. The restricted spectrum of cardiac anomalies in BMPRII^{DE2} mutants makes this strain a key murine model to understand the embryonic defects of Persistent Truncus Arteriosus, and impaired semilunar valve formation in humans.

Program Nr: 45 from the 2001 ASHG Annual Meeting

Role(s) of *Tbx1* in pharyngeal arch and cardiovascular development. *F. Vitelli, M. Morishima, A. Baldini.* Dept Pediatrics-Cardiology, Baylor College of Medicine, Houston, TX.

Tbx1 is the haploinsufficient gene responsible for aortic arch defects in the *Df1/+* mouse, a model for DiGeorge syndrome. We used a *Tbx1*-lacZ knock-in allele to compare *Tbx1* expression with phenotype in embryos with none, one or two functional *Tbx1* alleles. *Tbx1* +/- embryos have hypoplastic 4th pharyngeal arch arteries. *Tbx1* expression is strongest in the pharyngeal endoderm near the artery and is most closely associated with the vessel during its formation, before manifestation of the phenotype. This suggests a role for *Tbx1* in the initial growth/remodeling of the vessel, probably through a non-cell autonomous mechanism. *Tbx1*-/- embryos exhibit severe hypoplasia and no segmentation of the pharynx resulting in aplasia of the caudal arches and pouches. Indeed, *Tbx1* expression is highest in the pharyngeal endoderm, where it follows an antero-posterior, medio-lateral gradient that parallels the direction of pharyngeal growth and patterning. *Tbx1*-/- mice show conotruncal hypoplasia and lack of aortico-pulmonary (AP) septation. AP septum aplasia is secondary to lack of pharyngeal structures from which the septum must develop. *Tbx1*-lacZ activity also labels the external, muscular wall of the conotruncus, suggesting that *Tbx1* modulates conotruncal development. Conal septation is also absent, consistent with *Tbx1* expression. Neural crest (NC) marker analyses reveal essentially no overlap with *Tbx1*-positive cells. Mutants show proper migratory streams, suggesting that patterning abnormalities observed in post-migratory NC cells are secondary to lack of caudal pharyngeal structures. Since NC is not required for pharyngeal segmentation (which is abnormal in mutants) and since the outflow phenotype is evident prior to invasion by NC, these cells likely have a secondary role in the *Tbx1* mutant phenotype. Overall, our data suggest that *Tbx1* has multiple roles in pharyngeal and cardiovascular development, acting through both cell autonomous and non-cell autonomous mechanisms. We speculate that *Tbx1* drives pharyngeal apparatus development through endoderm-based signals, similar to endoderm-driven outgrowth of organs of the digestive and respiratory tracts.

Program Nr: 46 from the 2001 ASHG Annual Meeting

Novel murine models of Mafan syndrome provide insight into disease pathogenesis. *D.P. Judge[1], N.J. Biery[1], L. Myers[1], L.Y. Sakai[2], H.C. Dietz[1].* 1) Johns Hopkins Univ, Baltimore, MD; 2) Shriners Hospital, Portland, OR.

MFS is a systemic disorder of connective tissue caused by mutations in the gene encoding fibrillin-1 (*FBN1*). A dominant negative mechanism has been inferred based on dominant inheritance, multimerization of monomers, and paucity of matrix-incorporated fibrillin-1 in cell lines heterozygous for *FBN1* mutations (<50% of controls). This model fails to reconcile the classic phenotype in some patients with nonsense mutations and extremely low levels of mutant transcript and protein. Prior mouse models utilized hypomorphic *Fbn1* alleles with limited utility to address this issue. We have created a novel strain with a fully expressed murine allele harboring mutation C1039G. Heterozygotes have age-dependent vascular changes including mild disorganization of elastic lamellae but no aortic dissection or aneurysm formation by 9 months of age. Homozygous animals provide the first opportunity to definitively assess the fate of mutant cysteine-substituted protein, the most common class of human mutation. Cultured dermal fibroblasts synthesize, secrete and stably incorporate normal amounts of mutant fibrillin-1 in the extracellular matrix. At birth these mice show normal cardiovascular development. Cysteine-substituted fibrillin-1 supports the establishment of an aortic media with normal elastic fiber content and architecture. However, the homozygous mice die at 6 to 12 days after birth. Contrary to prior models, these data suggest that loss of function does not require a deficiency of microfibrils and occurs downstream of elastogenesis. YAC-based transgenesis was used to create mouse strains with regulated expression of both mutant (C1663R) and WT human fibrillin-1. IHC and co-immunoEM using Ab specific to human fibrillin-1 documented that the human protein is stably incorporated into extracellular microfibrils and interacts with mouse fibrillin-1. However, despite massive overexpression of either wt or mutant fibrillin-1, there was no discernible vascular phenotype. These data argue against a traditional dominant negative mechanism. The emerging view is that haploinsufficiency for a regulatory property of fibrillin-1 may contribute to disease pathogenesis in humans.

Program Nr: 47 from the 2001 ASHG Annual Meeting

Abnormal genome-wide methylation patterns in normal and uniparental early mouse embryos. *T.H. Haaf[1], W. Shi[1], R. Fundele[1], K.L. Arney[2], A.H. Surani[2], S.C. Barton[2].* 1) Max Planck Institute for Molecular Genetics, Berlin, Germany; 2) Cancer and Developmental Biology, Wellcome CRC Institute, Cambridge, UK.

In the initial 7-8 hours of the first G1 phase in normal diploid mouse embryos, active demethylation of the paternal genome but not of the maternal genome occurs in a highly coordinated fashion. Demethylation of the maternal genome occurs only after the two-cell embryo stage, using a passive replication-dependent mechanism. Genome-wide opposing patterns in paternal and maternal DNA methylation are believed to be fundamental for epigenetic reprogramming of the two parental genomes for somatic development. By nuclear transfer between fertilized eggs and subsequent immunofluorescence staining with an antibody against 5-methylcytosine, we show that the fertilized egg cannot demethylate the second maternal genome in diploid gynogenetic and triploid digynic embryos or remethylate the additional (already demethylated) paternal genome in androgenetic and triploid diandric embryos. The inability of the egg to demethylate the two maternal genomes in diploid parthenogenetic embryos demonstrates that the demethylation activity is restricted to paternal DNA, regardless of the number of genomes present. This suggests that differential zygotic demethylation results from differences in the remodeling of paternal and maternal chromatin structures after fertilization. During the period of protamine-histone exchange, the paternal DNA is unusually loosely packaged and, therefore, provides a unique opportunity for the binding of a demethylating enzyme(s). A significant proportion of embryos derived from superovulated normal matings and from in vitro fertilization display abnormal methylation patterns, some of which are indistinguishable from those in androgenetic or gynogenetic embryos. These embryos with disturbances in differential demethylation of the two parental genomes may be expected to fail early in development. Epigenetic reprogramming defects in the early diploid embryo may lead to spontaneous abortions during preimplantation development and also contribute to the high loss of embryos after in vitro fertilization.

Program Nr: 48 from the 2001 ASHG Annual Meeting

Targeted Deletion of Lymphocyte Specific Helicase (LSH) Results in Global Hypomethylation of the Mouse Genome. *K.E. Dennis[1], T. Fan[1], T. Geiman[1], Q. Yan[1], K. Muegge[2]*. 1) LMI, National Cancer Institute, Frederick, MD; 2) SAIC, LMI, National Cancer Institute, Frederick, MD.

Methylation of the mammalian genome is thought to be necessary for normal fetal development. Recent studies demonstrate that loss of methylation is embryonic lethal. At present however, the role of DNA methylation in chromatin regulation and embryonic development is not well understood. We are investigating the role of Lymphocyte Specific Helicase (LSH), a member of the SWI/SNF family of helicases, in chromatin regulation and specifically in genomic methylation. Our earlier reports have shown that targeted deletion of LSH from the mouse genome is perinatally fatal with no major aberrant morphological fetal development. Here we report that methylation is lost throughout the LSH-/- mouse genome. Southern Blot analysis shows a substantial loss of methylation at several repetitive sequences including both major and minor satellite repeats and retroviral inserts. All other genic sequences examined including imprinted and single copy genes, as well as genes on the inactivated X chromosome were found to be hypomethylated. We also report that quantitative assays of methylation indicate an overall loss exceeding 50% of normal methylation found within the mouse genome. These studies suggest that global methylation of the mouse genome is not essential for embryonic development to term. Moreover, LSH is necessary for normal methylation of the mouse genome and may possibly be involved in the regulation of chromatin organization.

Program Nr: 49 from the 2001 ASHG Annual Meeting

Targeted deletion of KvDMR1 results in growth retardation and loss of imprinting of multiple genes on mouse distal chromosome 7. *G.V. Fitzpatrick, P.D. Soloway, M.J. Higgins.* Roswell Park Cancer Inst, Buffalo, NY.

Imprinted genes are preferentially expressed from either the maternal or paternal allele and deregulation of imprinting have been implicated in several developmental disorders. The imprinting disorder Beckwith-Wiedemann syndrome can arise from a variety of genetic and epigenetic mechanisms affecting imprinted genes in human chromosome 11p15.5 (mouse distal chromosome 7) including inactivating mutations of *CDKN1C* (*p57KIP2*). In more than 50% of non-UPD (uniparental disomy) sporadic BWS patients, the disease is associated with the loss of methylation (LOM) from the maternal allele of KvDMR1, a differentially methylated CpG island within the *KCNQ1* gene. It has been proposed that differentially methylated CpG islands might function as imprinting control regions (ICRs). In order to test if KvDMR1 is an ICR, we generated mouse lines carrying a 2.5-kb deletion of this locus. Embryos carrying a paternal deletion of KvDMR1 exhibit a 20% reduction in weight compared to wild-type littermates. There is no effect on growth when the deletion is present on the maternal chromosome. Using RT-PCR, the effect of this deletion on imprinted gene expression was assessed in tissues of F1 fetuses from crosses between deletion-carrying animals and PWK mice. Following paternal transmission, the KvDMR1 deletion resulted in biallelic expression of *Orctl2*, *Cdkn1c*, and *Kcnq1* (*Kvlqt1*), genes usually transcribed only from maternally derived chromosome. In contrast, normal maternal-specific expression of these genes was observed in mutant mice which inherited the deletion from their mother. These results suggest that the unmethylated KvDMR1 locus regulates imprinted expression by repressing genes on the paternal chromosome. We have also shown that murine KvDMR1 functions as a chromatin insulator in a cell culture assay (Higgins and Fitzpatrick, this meeting). We propose that KvDMR1 is an ICR which silences genes on the paternal chromosome by functioning as a chromatin insulator, similar to the *Igf2/H19* ICR. These data support the notion that LOM at KvDMR1 on the maternal chromosome results in the repression of *CDKN1C* expression which leads to BWS. Funded by NIH/NCI Grant CA63333.

Program Nr: 50 from the 2001 ASHG Annual Meeting

Genome wide linkage analysis assessing parent-of-origin effects in the inheritance of birth weight. *R.S. Lindsay, S. Kobes, W.C. Knowler, R.L. Hanson.* NIDDK, NIH, Phoenix, AZ.

Family studies suggest that genetic variation may influence birth weight. We have assessed linkage of birth weight (adjusted for gestational age and gender) in a genome wide scan in 172 Pima siblings (177 sibling pairs, 66 families). As imprinting (expression of only a single copy of a gene depending on parent-of-origin) is commonly found in genes which affect fetal growth, we used a recently described modification of multipoint variance-component methods of linkage analysis of quantitative traits. This technique allows assessment of linkage models in which parent-of-origin effects are either incorporated (strength of linkage expressed as LOD_{IMP}) or not (LOD_{EQ}). Where significant evidence of linkage was present, separate contributions of paternally (LOD_{FA}) and maternally (LOD_{MO}) derived alleles within the imprinting model were estimated.

Significant evidence of linkage was found on chromosome 11p (at map position 33cM, LOD_{IMP}=3.5, LOD_{EQ}=0.8) and chromosome 15 (at 50cM, LOD_{IMP}= 3.8, LOD_{EQ}=2.8), with evidence for imprinting in both regions (imprinting model superior, $P<0.005$). Birth weight was linked predominantly to paternally derived alleles on chromosome 11p (LOD_{FA}= 4.2, LOD_{MO}= 0.0) and to maternally derived alleles on chromosome 15 (LOD_{FA}= 0.0, LOD_{MO}=4.4).

We conclude that imprinted genes on chromosomes 11p and 15 may influence birth weight in the Pima population. These chromosomes contain the two major, known clusters of imprinted genes in the human genome, lending biological plausibility to our findings.

Program Nr: 51 from the 2001 ASHG Annual Meeting

Evolutionary significance of the regulation of *Snrpn* by its upstream open reading frame. *K.-S. Chen, T.-F. Tsai, J.S. Weber, M.J. Justice, A.L. Beaudet.* Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX.

In order to isolate mutations affecting genomic imprinting related to Prader-Willi and Angelman syndromes, we produced a fusion transgene in the mouse with the imprinted *Snrpn* promoter driving an agouti coat color reporter gene. The *Snrpn*/agouti fusion resulted in a tannish abdomen when inherited paternally, but had no visible effect on the C57BL/6 black color when inherited maternally. Differential methylation and imprinted expression of the *Snrpn* promoter were preserved and switched normally. Screening of ENU mutagenized wild type males mated to homozygous transgenic females identified one mouse with an Angelman-like imprinting defect with loss of silencing of the fusion gene, abnormal methylation at *Snrpn* and *Ndn*, obesity, and infertility, but no detectable deletion of the imprinting center. Screening of ENU mutagenized homozygous transgenic males mated to wide type females identified one mouse with an ATG to AAG mutation in the initiation codon of the upstream open reading frame (ORF) of the normally bicistronic Snrpn transcript. The ATG to AAG mutation resulted in an approximately 15-fold decrease in translation of the SmN protein. These results demonstrate the feasibility of using a gene fusion strategy to screen for regulatory mutations. We hypothesize that the upstream ORF of the *Snrpn* transcript, that is highly conserved between mouse and human, has been selected evolutionarily in whole or in part for its ability to regulate the translation of the ORF for SmN.

Program Nr: 52 from the 2001 ASHG Annual Meeting

Imprint establishment in Prader-Willi/Angelman syndromes is controlled by NRF-1 and CTCF transcription factors at alternative promoters of *SNURF-SNRPN*. *T. Ohta[1], J.R. Khadake[2], S. Rodriguez-Jato[2], C. Mione[2], J.L. Knepper[1], P. Svoboda[1], J.R. McCarrey[3], R.M. Schultz[1], T.P. Yang[2], R.D. Nicholls[1].* 1) Univ. Pennsylvania, Philadelphia; 2) Univ. Florida, Gainesville; 3) Southwest Found., San Antonio, TX.

Mutations in Prader-Willi (PWS) and Angelman (AS) syndrome imprinting defects (IDs) occur at the *SNURF-SNRPN* (*S-S*) exon 1-promoter (e1p) or 25 kb 5' of this, respectively. These mutations define genetic elements controlling imprinting for a 2 Mb domain in human chromosome (chr) 15q11-q13. IDs in PWS lead to a maternal (mat) imprint on the paternally derived chr due to a failure to maintain the paternal (pat) imprint postzygotically. In contrast, the basis of AS IDs is unknown. One molecular model for imprinting involves transcription factor (TF) competition between *S-S* promoters at e1 or upstream (U) alternative starts at eU1a/eU1b. Here, we provide support for this model in the human and homologous mouse chr 7C imprinted domains. Alternatively spliced upstream *S-S* transcripts were identified in mouse, but they show no homology to those in human. Surprisingly, the mouse eU1 and promoter are duplicated 9 times, deriving from *Snurf* e1p-e1, and exhibiting imprinted, pat-only expression. Only 2 CpG dinucleotides occur in most eU1 promoters, and these show differential allelic methylation in brain. Both CpGs occur in a recognition site for a methylation-sensitive TF, NRF-1, suggesting that NRF-1 controls imprinted transcription from U1 promoters. Adjacent to this site is a putative motif for CTCF, with both conserved in human eU1p and with similar motifs in mammalian *S-S* e1p. *In vitro* EMSAs show NRF-1 binds these sites. Dimethyl sulfate *in vivo* footprinting confirms TF occupancy at the NRF-1 and putative CTCF sites in mouse e1p in brain. Mouse transcripts from eU1p are detected in brain, early preimplantation embryos (pe) and female germ cells, while *S-S* is expressed in late pe but not female germ cells, with male germ cells under study. These data suggest that TF binding and transcription from eU1p in oocytes controls switching of the pat to mat imprint while similar events at e1p maintain the pat imprint postzygotically.

Program Nr: 53 from the 2001 ASHG Annual Meeting

Variable X inactivation of the human TIMP1 gene is due to instability of silencing associated with altered histone acetylation. *C.L. Anderson, C.J. Brown.* Dept of Medical Genetics, University of British Columbia, Vancouver, BC, Canada.

Stable transcriptional silencing is critical for normal development, and is associated with several epigenetic modifications. The human TIMP1 gene offers a unique system to examine these epigenetic features since it has variable X inactivation, being expressed from some inactive X (Xi) chromosomes but silenced on others. We examined methylation and expression patterns in human and somatic cell hybrid cell lines and found that TIMP1 was generally unmethylated when expressed, except in 2 of 3 Xi-containing hybrids expressing TIMP1. Single cell clones of these hybrids demonstrated that methylation was associated with instability. From a methylated TIMP1+ culture, only 58% of clones remained TIMP1+ whereas all subclones from an unmethylated culture retained expression. Methylated TIMP- cultures were prone to expression as 1/13 subclones reactivated TIMP1. This contrasts with the stable silencing observed in Xi hybrids derived from females inactivating TIMP1 and suggests another epigenetic feature differs in the Xi hybrids expressing TIMP1.

As hypoacetylation of histones correlates with stable inactivation, we performed ChIP with antibodies to acetylated H3 followed by PCR of promoter regions. TIMP1 was hyperacetylated when expressed, but not in hybrids that had never expressed TIMP1. Intriguingly, when the clones from the Xi hybrids that expressed TIMP1 were examined, the TIMP1 promoter was always hyperacetylated regardless of current expression status. Acetylation of histone H3 is therefore associated with the ability of TIMP1 to express from the inactive X chromosome. To determine if this was a reflection of chromatin configuration, we assayed DNaseI sensitivity. In cell lines with stable TIMP1 expression, the promoter was nuclease sensitive, suggesting an open chromatin conformation. The promoter remained nuclease insensitive in all silent clones, similar to the methylation results. These results establish a hierarchy to the epigenetic silencing of TIMP1, with histone acetylation preceding expression whereas methylation and chromatin structure are concordant with expression.

Program Nr: 54 from the 2001 ASHG Annual Meeting

The histone variant macroH2A is enriched at the inactive X chromosome and the centrosome in a cell cycle-dependent manner in female somatic cells. *B.P. Chadwick, H.F. Willard.* Dept Genetics, BRB 701, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, OH.

Chromatin of the inactive X chromosome (Xi) in female mammals is enriched for the core histone variant macroH2A. MacroH2A is evenly distributed throughout the male nucleus, while in female nuclei macroH2A additionally is enriched on chromatin of the Xi, and forms a structure referred to as a macro chromatin body (MCB) that is coincident with the Barr body. X-inactivation studies in differentiating female mouse embryonic stem cells demonstrate that macroH2A relocates from a centrosomal accumulation in the cytoplasm to chromatin of the Xi after the initial stages of X-inactivation have occurred (Rasmussen et al., 2000. JCB 150, 1189-1198). We have investigated the cellular distribution of macroH2A during the cell cycle in synchronized cultured human female somatic cells. As the cells approach mitosis, the MCB (as detected by indirect immunofluorescence using anti-macroH2A antibodies) decreases dramatically in size to a small chromatic body (SCB). Interestingly, as the MCB shrinks in size, macroH2A begins to accumulate at the centrosome. During mitosis, macroH2A remains associated with specific regions of the Xi, with the most enrichment at Xq22-25. After mitosis, the levels of macroH2A at the centrosome decrease as the MCB reforms at the Xi. We investigated the timing of MCB dissipation and reformation in relation to the timing of XIST RNA association with the Xi. XIST RNA remains associated with the inactive X chromatin until after MCB dissipation, before becoming diffusely centered at the SCB. During mitosis, XIST RNA does not remain associated with the Xi. Shortly after mitosis, however, a discrete focus of XIST RNA can be observed that begins to accumulate with the Xi centered at the SCB. After XIST RNA has stably associated with the inactive X chromatin, an MCB begins to form coincident with dissociation of macroH2A at the centrosome. We hypothesize that the enrichment of macroH2A at Xq22-25 may act as a nucleation site for the reassociation of XIST RNA and that the centrosome acts as a storage site for non-nucleosomal macroH2A during mitosis.

Program Nr: 55 from the 2001 ASHG Annual Meeting

Influence of X-chromosome inactivation on Rett syndrome phenotypes. *T. Kubota[1], Y. Nomura[2], K. Amano[3], K. Oshina[1], K. Yamakawa[3], M. Segawa[2], Y. Goto[1]*. 1) Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, NCNP, Tokyo; 2) Segawa Neurological Clinicfor Children, Tokyo; 3) Laboratory for Neurogenetics, Brain Science Institute, RIKEN, Saitama, Japan.

Rett syndrome (RTT) is an X-linked dominant disease caused by mutation of MeCP2 gene. The phenotypes of this disease are thought to be determined by mutation site in combination with an X-chromosome inactivation (XCI) pattern. However, little is known about the influence of the XCI pattern on the phenotype. Therefore, we examined XCI patterns using an assay based on methylation-specific PCR, as well as mutations of MeCP2 gene. Out of 31 RTT patients analyzed, a mutation was detected in 23 patients (74%): 10 were found in the methyl-CpG binding domain (MBD), 12 in the transcriptional repression domain (TRD), and 1 in between these two domains. Application of a development-scoring system newly developed (Min. 0; Max. 8) based on 4 key milestones of this disease revealed that patients with a mutation in the MBD was more severely retarded in motor and metal development than those with a mutation in the TRD (average 4.2 vs. 6.3, respectively; $p<0.05$). Of the 23 patients with a mutation, XCI patterns were obtained in 19 patients. Of these, an extremely non-random (NR)-XCI pattern (>90:10) was demonstrated in 3 patients: one with a mutation in the MBD (R168X) showed an exceptionally mild phenotype with the presence of speech (score 8), compared with 2 patients with the same mutation and a random (R)-XCI pattern (average score 3.5); one with a deletion in the TRD showed a severe phenotype with lack of speech (score 5), compared with 3 patients with a similar deletion and an R-XCI pattern (average score 7.3). These results indicate that a mild phenotype of the former patient and a severe phenotype of the latter patient may be caused by a favorable and an unfavorable NR-XCI pattern, respectively. These findings imply that an XCI pattern can modify the RTT phenotypes that are initially determined by the mutation site of MeCP2 gene.

Program Nr: 56 from the 2001 ASHG Annual Meeting

Mutations in the gene for epsilon-sarcoglycan (SGCE) cause myoclonus-dystonia syndrome. *M. Grabowski[1,2], A. Zimprich[3], F. Asmus[3], M. Bauer[3], M. Naumann[4], D. Berg[4], M. Bertram[5], J. Winkelmann[6], B. Müller-Myhsok[7], T. Meitinger[1,2], T.M. Strom[1,2], T. Gasser[3]*. 1) Institute for Human Genetics, GSF National Research Center, Neuherberg, Germany; 2) Institute for Human Genetics, Klinikum rechts der Isar, Technical University Munich, Germany; 3) Department of Neurology, Klinikum Grohadern, Ludwig-Maximilians-University, Munich, Germany; 4) Department of Neurology, University of Wrzburg, Germany; 5) Department of Neurology, University of Heidelberg, Germany; 6) Max-Planck-Institute for Psychiatry, Department of Neurology, Munich, Germany; 7) Bernhard-Nocht for Tropical Medicine, Hamburg, Germany.

Myoclonus-dystonia syndrome (MDS, DYT11) is an autosomal-dominant disorder characterized by bilateral, alcohol-sensitive myoclonic jerks, involving predominantly the arms and axial muscles. Dystonia (usually cervical dystonia and/or writer's cramp) occurs in most, but not all affecteds and may occasionally be the only symptom of the disease. Furthermore, patients often show prominent psychiatric abnormalities, including panic attacks and obsessive-compulsive behavior. In the majority of MDS families, the disease is linked to a locus on chromosome 7q21. Using a positional cloning approach, we have identified five different heterozygous loss-of-function mutations in the epsilon-sarcoglycan gene, which mapped to a refined critical region of about 3.5 Mb. This is the fourth inherited dystonia gene cloned and the first to involve a structural protein. Pedigree analysis shows a marked difference in penetrance depending on the parental origin of the disease allele. This is suggestive of a maternal imprinting mechanism which has been demonstrated in the mouse epsilon-sarcoglycan gene.

Program Nr: 57 from the 2001 ASHG Annual Meeting

Optimized gene activation utilizing artificial transcription factors. *R. Yaghmai, G. Cutting.* McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins Hospital, Baltimore, MD.

A major focus in the study of regulation of eukaryotic gene expression is the ability of various transcriptional effectors (activators or repressors) to differentially influence the assembly of the transcription preinitiation complex (PIC) at the core promoter of genes in different cell types. We engineered transcription factors using designed polydactyl zinc finger proteins fused to various transcriptional effector domains derived from the naturally occurring activators VP16 or P65. Here we report the effect of targeting these transcription factors to different positions and orientations with respect to the SV40 core promoter element. Our results reveal that factors containing six zinc finger domains have improved target site affinities and are more potent activators than factors with three finger domains. Furthermore, the extent of activation depends on the nature of the activator domain and the cell type used. Finally, the position of the target site with respect to the core promoter can substantially influence the extent of transcription activation. The most effective activation occurred using factors targeted just upstream of the PIC assembly site which suggests a novel approach to functionally locate endogenous core promoter elements. These observations support the recently emerging notion that activated transcription is not only a result of recruitment of factors to a given promoter, but also the result of efficient PIC assembly in the correct orientation for transcription.

Program Nr: 58 from the 2001 ASHG Annual Meeting

Identification of molecular partners of the TRPS1 transcription factor. *F. Kaiser[1], K. Tavassoli[2], T. Möröy[2], B. Horsthemke[1], H.-J. Lüdecke[1]*. 1) Institut für Humangenetik, Universitätsklinikum, Essen, Germany; 2) Institut für Zellbiologie, Universitätsklinikum, Essen, Germany.

Mutations in the *TRPS1* gene cause tricho-rhino-phalangeal syndrome, which is characterized by craniofacial and skeletal abnormalities. The TRPS1 protein is a 1281 amino acids nuclear transcription factor with multiple different zinc-finger motifs, including a DNA-binding GATA zinc-finger and a putative protein interacting IKAROS-like domain. We used the C-terminal 647 amino acids of the highly identical (93 %) murine Trps1 in a yeast-two-hybrid screen in order to identify interacting partners. We identified six different proteins. The interactions were verified by *in vitro* GST-assays and *in vivo* GAL1-assays. Experiments with truncated Trps1 proteins narrowed down the interaction domains and excluded the DNA binding GATA zinc-finger. So far, we studied in more detail the interactions of two partners of TRPS1, the nuclear RING finger protein 4 (RNF4 or small nuclear RING finger, SNURF) and a cytoplasmic protein with 89 amino acids known as protein inhibitor of nNOS (PIN) or dynein light chain 8 (DLC8). Ectopic TRPS1 could be co-immunoprecipitated with both partners from COS cell extracts. TRPS1 co-localizes with RNF4 in the nucleus of NIH3T3 cells. Interestingly, we found that most of PIN/DLC8, which is a cytoplasmic molecule, is translocated into the nucleus when it is coexpressed with TRPS1 in these cells. The interaction of TRPS1 with RNF4 could be narrowed down to the 65 N-terminal amino acids of RNF4. This part of RNF4 contains the nuclear localization signal and part of the androgen receptor interacting domain. This is interesting because TRPS1, also known as GC79, is upregulated in androgen sensitive prostate cancer cells which undergo apoptosis upon androgen withdrawal. Beside many other functions, PIN/DLC8 has been implicated in apoptosis regulation through inactivation of the Bcl-2 family member Bim. Our results suggest that the complex phenotype of TRP syndrome may be caused by errors in apoptosis pathways e.g. in cartilage development and hair follicle morphogenesis and cycling.

Program Nr: 59 from the 2001 ASHG Annual Meeting

Defining cis-acting regulatory elements by transgenic BAC scanning and comparative sequencing: examples from the GDF/BMP gene family. *D.P. Mortlock[1], C. Guenther[1], M. Schoor[2], D.M. Kingsley[1]*. 1) Dept. of Developmental Biology, Stanford University, Stanford, CA; 2) Artemis Pharmeceuticals.

Cis-acting regulatory elements that direct gene expression are critical for developmental patterning, differentiation, and health. Despite much progress in predicting transcribed regions within genomic sequence, efficient methods for identifying regulatory sequences in genomic DNA are still lacking. This problem is compounded by the tendency of cis-regulatory elements to be separated from target promoters by large distances. We have used BAC transgenic analysis and comparative sequencing to identify regulatory control regions that pattern developing limb joints as well as numerous other skeletal components and soft tissues. A "BAC scanning" transgenic reporter method was first used to localize distant regulatory regions flanking two genes (Gdf5 and Gdf6) expressed in different subsets of mouse limb joints. For both genes, key control elements that drive in vivo expression were found far from their promoters, demonstrating the utility of BAC scanning to survey regulatory information around each locus. In a second round of analysis, large-scale comparisons were performed to identify blocks of conserved non-coding sequence flanking mouse and human Gdf5, Gdf6 and Bmp5 genes. Numerous conserved noncoding regions were found within the different regulatory control regions defined by BAC transgenes. Finally, homologous recombination in bacteria was used to modify conserved sequence blocks within BAC transgene constructs, allowing tests of whether conserved regions identified by mouse/human sequence comparisons are predictive of true regulatory elements. These studies have further defined critical regulatory regions and identified specific short conserved sequences that may influence GDF/BMP patterning during skeletal development. These data also demonstrate the utility of combining BAC transgenics with large-scale sequence comparison, and the richness of regulatory information within the intergenic DNA of mammalian genomes. A similar approach should be widely applicable to the study of many other vertebrate genes.

Program Nr: 60 from the 2001 ASHG Annual Meeting

***RUNX2/CBFA1* regulation of type X collagen gene expression contributes to abnormal endochondral ossification observed in cleidocranial dysplasia.** *Q. Zheng[1], G. Zhou[1], Y. Chen[1], D. Krakow[2], W. Wilcox[2], B. Lee[1]*. 1) Molec & Human Genetics, Baylor College Med, Houston, TX; 2) Dept. Pediatrics, Cedar Sinai Medical Center, Los Angeles, CA.

Core binding factor alpha-1 (*Runx2/cbfa1*) is an essential transcriptional determinant of osteoblast differentiation. Its haploinsufficiency in humans causes alteration in intramembranous ossification observed in dominantly inherited cleidocranial dysplasia (CCD). However, CCD patients also have short stature and clinical features suggesting the presence of long bone dysplasia, and hence, defective endochondral ossification. Here, we demonstrate that defective terminal chondrocyte hypertrophy is present in CCD and is due in part to decreased *cbfa1* transactivation of the hypertrophic chondrocyte-specific type X collagen gene (*Col10a1*). We demonstrate that *Cbfa1* can transactivate a minimal *Col10a1* promoter via highly conserved *Cbfa1* binding sites found in its proximal 4 kb promoter. Also, transfection of SV40 T antigen immortalized mouse chondrocytes (MCT cells) which hypertrophy at 37C, but not at 32C with a 4kb proximal *Col10a1* promoter containing the Cbfa1-binding sites directed 50-100 fold greater reporter activity upon growth arrest and hypertrophy (culture at 37C). Moreover, transgenic mice studies show that this same promoter can drive b-galactosidase expression in hypertrophic chondrocytes, but not in resting or proliferative chondrocytes. When the same reporter is fixed on a *Cbfa1* heterozygote background, decreased staining was observed in the hypertrophic zone. Finally, we correlated these findings by finding decreased type X collagen expression in a markedly shortened zone of hypertrophy in the growth plate of a fetal case of CCD. Therefore, we identify *Col10a1* as one of several transcriptional targets of *Cbfa1* during chondrogenesis and provide a mechanistic basis for the pathogenesis of long bone dysplasia in CCD. Together these data provide a molecular link between the processes of chondrocyte hypertrophy and osteoblastogenesis.

Program Nr: 61 from the 2001 ASHG Annual Meeting

Genetic mechanisms of age regulation of pro- and anti-blood coagulation factors. A**.** *K. Kurachi, K. Zhang, S. Kurachi.* Dept Human Genetics, Univ Michigan Medical Sch, Ann Arbor, MI.

Blood coagulation capacity in normal humans increases with advancing age. This phenomenon has substantial clinical importance. We recently determined for the first time the age-regulatory mechanisms of the hFIX gene, identifying two critical genetic elements, ASE (AE5') and AIE (AE3') (Science 1999;285:739-743). ASE and AIE are essential for the age-dependent stability and increase of hFIX gene expression, respectively, and together can recapitulate the age-dependent regulation of the natural hFIX gene. Through systematic and longitudinal testing of transgenic mice carrying minigenes of human anti-blood coagulation protein C (hPC), we now have established the basic genetic mechanisms of age regulation of the hPC gene. Age-stable expression observed for the natural hPC gene critically depends on an ASE consensus element (hPC-ASE) present in the 5' flanking upstream. Unlike the hFIX gene, the hPC gene lacks AIE, thus, unlike hFIX, lacking age-dependent increase in its expression. All the hPC minigenes without hPC ASE show age-unstable hPC expression in transgenic mice, but when hFIX-ASE was added, they reproducibly showed age-stable expression patterns. Furthermore, addition of hFIX-AIE converted the age-stable expression patterns of hPC minigenes with hPC ASE to age-dependent increase patterns. These experiments proved the functional universality of these elements and the regulatory mechanisms involving them. The 5' flanking upstream region of hFIX, where hFIX-ASE is present, was derived from retrotransposed LINE-1, whereas the corresponding 5' flanking region of the hPC gene is not. Furthermore, hPC and hFIX genes share no significant similarity in their 3' UTRs. These facts strongly support that the age-regulatory mechanisms of both pro and anti-coagulation factor genes were generated through convergent evolution. We now have established the fundamental molecular mechanisms responsible for age-regulation of both pro and anti-coagulant factors, thus laying the foundation for comprehensive understanding of age-associated regulation (issue of homeostasis) of the blood coagulation system.

Program Nr: 62 from the 2001 ASHG Annual Meeting

Quantitative modulation of the Tyrosine Hydroxylase gene expression by the HUMTH01 microsatellite: implications for complex genetic traits. *R. Meloni[1], T. Aranyi[1], V. Albanese[1], O. Khalfallah[1], H. Kiefer[1], B.A. Faucheux[2], N. Faucon Biguet[1], J. Mallet[1]*. 1) LGN-CNRS, Bat CERVI, Hopital de la Pitie-Salpetriere, 75013 Paris, France; 2) INSERM U. 289, Hopital de la Pitie-Salpetriere, 75013 Paris, France.

A polymorphic (TCAT)n microsatellite (HUMTH01) is located in the first intron of the tyrosine hydroxylase (TH) gene. This microsatellite is widely used in genetic, population and forensic studies and has been found associated with several complex genetic traits. We have previously shown that alleles of this microsatellite act as transcriptional elements under the drive of a heterologous minimal promoter and are recognized by specific transacting factors. Thus, we evaluated by means of transient transfection assays the HUMTH01 microsatellite in the context of the TH gene. The microsatellite alleles differentially modulate the TH gene activity in a quantitative mode. We then cloned by the yeast simple hybrid system a transacting factor that exhibits specific affinity for the HUMTH01 sequence correlated to variations in the number of (TCAT)n repetitions. The transcriptional activity of the HUMTH01 microsatellite appears to be independent of the TH promoter but conditioned by its surrounding sequences. Thus, we investigated using the bisulfite sequencing method the methylation profile of a CpG island flanking the repeated sequence to evaluate the role of epigenetic modifications in the transcriptional regulation of the TH gene. TH+ and TH- human cell lines and single cells obtained by Laser Capture Microdissection exhibited differential methylation patterns in the first exon of the TH gene. We then identified by gel shift assay a new putative Ap2 site in this region. The (TCAT)n polymorphic sequence is not limited to the TH gene but is widespread throughout the genome. Thus, the characterization of the transduction pathway impinging on the HUMTH01 microsatellite and its flanking sequences may be relevant for the transcriptional regulation of the TH gene as well as that of other genes implicated in normal and pathological complex genetic traits.

Program Nr: 63 from the 2001 ASHG Annual Meeting

Altered androgen receptor function caused by polyglutamine expansion. *A.P. Lieberman[1], G. Harmison[1], A. Strand[2], J. Olson[2], K.H. Fischbeck[1]*. 1) Neurogenetics Branch, NINDS/NIH, Bethesda, MD; 2) Fred Hutchinson Cancer Research Center, Seattle, WA.

Eight neurodegenerative diseases are caused by expansions of CAG/polyglutamine tracts within the coding regions of disease-causing genes. Among these disorders is Kennedys disease, a degenerative disease of motor neurons in which the causative mutation occurs near the 5 end of the androgen receptor gene. The mutant protein misfolds, aggregates, and abnormally interacts with other proteins, leading to both a toxic gain-of-function and a loss of normal function. Motor neurons, which express the androgen receptor and are targets of degeneration in Kennedys disease, also respond to activation of the receptor by showing trophic effects. We used a motor neuron cell culture model to explore the mechanisms underlying these various effects of the wild-type and mutant receptors. Cells expressing the wild-type androgen receptor with 24 CAG repeats respond to ligand by showing trophic effects including prolonged survival in low serum, whereas cells expressing the mutant receptor with 65 CAG repeats do not show a robust trophic response. This partial loss-of-function correlates with decreased accumulation of the mutant protein due to its rapid degradation by the lactacystin-sensitive proteasome pathway. Expression analysis using oligonucleotide arrays confirms that the mutant receptor has undergone a partial loss-of-function, and fails to regulate a subset of genes whose expression is normally altered by ligand activation of the wild-type receptor. The mutant receptor has also undergone several functionally important post-translational modifications in the absence of ligand that the wild-type receptor undergoes in the presence of ligand, including acetylation and phosphorylation. These alterations correlate with a ligand independent gain-of-function exhibited by the mutant receptor in expression analysis. Our findings suggest that polyglutamine expansion alters androgen receptor function by promoting its rapid degradation and by modifying its activity as a transcription factor.

Program Nr: 64 from the 2001 ASHG Annual Meeting

Insights into psoriasis and other inflammatory diseases from gene expression studies. *A.M. Bowcock[1], W. Shannon[1], F. Du[1], J. Duncan[1], K. Cao[2], K. Aftergut[3], J. Catier[3], M. Fernandez-Vina[2], A. Menter[3].* 1) Washington Univ, St Louis, MO; 2) Georgetown University, Kensington, MD; 3) Baylor University Medical Center, Dallas, TX.

Approximately 2% of the Caucasian population are affected by psoriasis (PS), a chronic inflammatory skin disease triggered by both genetic and environmental risk factors. In addition to a major contribution from the HLA class I region, PS susceptibility loci have been mapped to 1q21, 3q21, 4qter, 14q31-q32, 16p, 17q23-q25, 19p13.3 and 20p. Some of these regions overlap with loci implicated in other autoimmune/inflammatory diseases. Global gene expression studies are beginning to provide insights into the etiology of these and other complex diseases. We used Affymetrix oligonucleotide arrays comprising 12,000 known genes to initiate a comprehensive analysis of the transcriptional changes that occur in PS. RNA was prepared from involved and uninvolved skin of fifteen psoriatic patients and six normal controls and hybridized to the arrays. Transcript levels detected on the arrays were first used to determine the relationship of samples to each other. A tree constructed with average linkage clearly differentiated involved psoriatic skin from uninvolved and normal skin. Differentially expressed genes were then identified with K-means clustering. This placed transcripts into clusters if they had similar expression levels in the same samples. This approach identified 6 clusters (a total of 177 genes) that were differentially expressed in involved psoriatic skin versus normal skin. These differences were independent of the gender, age, skin site and HLA class I status of the patient. Reclustering of the samples with just these 177 genes more definitely separated the involved from uninvolved and normal samples, indicating that these genes are likely to have real relevance to PS. Ten of the 177 genes were also differentially expressed in uninvolved skin and several mapped to regions previously shown to harbor PS susceptibility loci. These included genes from the Epidermal Differentiation Complex on chromosome 1q21 and a novel up-regulated gene: interferon-alpha inducible protein 27 at 14q31-q32.

Program Nr: 65 from the 2001 ASHG Annual Meeting

Large scale gene expression profiling of early human craniofacial development. *Y. Korshunova[1], R. Tidwell[1], N. Fukushima[1], D. Messina[1], C. Helms[1], M. Lovett[1], T. Attié-Bitach[2], J. Augé[2], S. Audollent[2], M. Vekemans[2], J. Cai[3], E.W. Jabs[3]*. 1) Washington University, St. Louis, MO; 2) Hopital Necker Enfants Malades, Paris; 3) The Johns Hopkins University, Baltimore, MD.

The Craniofacial and Oral Gene Expression Network (COGENE) is measuring changes in gene expression during early human development with a particular emphasis on craniofacial structures. Starting with small quantities (~500ng) of total RNA we developed or adapted three complementary expression profiling methods (normalized micro-cDNA libraries, micro-SAGE, and Affymetrix gene chips) to enhance the reliability of our measurements. To date we have generated gene chip expression profiles from a total of 21 different microdissected craniofacial structures that are of particular importance in the development of the head and face from weeks four through eight of human embryogenesis. We constructed normalized cDNA libraries from 8 of these structures and have derived SAGE tags from 4 of them. Hierarchical clustering of our gene chip data reveals a strong similarity in expression profiles between structures from the same developmental stage, and more significant changes occurring within the same structure at different times during development. For example, the expression profiles of the 1st and 2nd pharyngeal arches at week four of development are more closely related to each other than they are to 1st and 2nd pharyngeal arches at week five of development. Comparisons of changes between sequential stages, such as the 1st pharyngeal arch at weeks four and five and the mandiblular and maxillary prominences at week six reveal > 4-fold changes in a set of 26 genes that includes the paired mesoderm homeobox 2b and forkhead box F2 genes. A similar comparison conducted on >4-fold changes occurring in the frontonasal prominence between weeks four and five of development identifies a cluster of genes that includes the fibroblast growth factor receptor 3 gene. The identification and validation of these changes should provide new insights into the genetic control of human head and face development. Primary data from the COGENE project is available online at http://hg.wustl.edu/COGENE/index.html.

Program Nr: 66 from the 2001 ASHG Annual Meeting

Differential gene expression in human preeclampsia. *L.M. Pierce[1], B.T. Pierce[1], B.C. Calhoun[1], R.F. Hume[1,2], L.S. Martin[1,2]*. 1) Madigan Army Med Ctr, Tacoma, WA; 2) Rockford Health System, IL.

The epigenetic and molecular mechanisms underlying the pathogenesis of preeclampsia are complex and suggest synergistic heterozygosity, modifier genes and/or unique feto-maternal signal transduction pathway interactions. Application of innovative genomic technology may identify which genes are upregulated or downregulated in preeclampsia providing potential molecular targets for intervention.

Placental tissue from both severe preeclampsia and normal pregnancy underwent differential gene expression analysis using the Atlas^{TM} cDNA Expression Array technology (Clontech). cDNA probes prepared from RNA isolated from placental tissue were hybridized to an array of 1,176 human genes involved in a wide range of biological pathways. Differential mRNA expression will be confirmed by banked normal and preeclamptic placentas stratified by clinical severity via Northern analysis or RT-PCR.

Initial analysis identified several genes involved in cell cycle regulation, signal transduction, and cell growth to be differentially expressed in placental tissues from preeclamptic compared to normal pregnancy. The expression of the gene encoding the katanin p80 subunit, an ATPase that disassembles microtubules during the rapid reorganization of the cytoskeleton during the cell cycle, differentiation, and cell migration, was decreased 18-fold in preeclampsia. The early growth response protein 1 was downregulated 3-fold in preeclampsia. Expression of teratocarcinoma-derived growth factor 1, which activates components of the ras/raf/MEK/MAPK signal transduction pathway, was decreased in preeclampsia almost 7-fold. Genes upregulated in preeclampsia included insulin-like growth factor binding protein 1 (increased 10-fold), which may inhibit the action of insulin-like growth factors at the endometrial-trophoblastic interphase, and pregnancy-associated major basic protein (increased 4.5-fold), a potent cytotoxin.

Preliminary results show promise for the discovery of differentially expressed genes that may be involved in critical cellular pathways for the pathogenesis of preeclampsia.

Program Nr: 67 from the 2001 ASHG Annual Meeting

Human bone mass accrual is affected by mutations in the low density lipoprotein receptor-related protein 5 gene (*LRP5*). *Y. Gong[1], R. Slee[1], Osteoporosis-Pseudoglioma Collaborative Group[2]*. 1) Department of Genetics and Center for Human Genetics, Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, OH; 2) An international consortium of clinicians and researchers.

Low bone mass is a risk factor for osteoporotic fractures. Osteoporosis-Pseudoglioma syndrome (OPPG) is an autosomal recessive, childhood-onset, bone fragility disorder characterized by low bone mass and visual impairment. We report that mutations in the low density lipoprotein receptor-related protein 5 gene (*LRP5*) are the cause of OPPG. We have found 8 different homozygous disease-causing nonsense or frameshift mutations in affected offspring of consanguineous unions. Because we found disease-causing mutations within the signal peptide at the amino-terminus and near the transmembrane domain at the carboxy-terminus we conclude that the OPPG phenotype results from a loss of LRP5 function. Using *in situ* hybridization, we found that LRP5 is expressed in osteoblasts. We also found that the expression level of LRP5 mRNA increased as mesenchymal cell lines differentiated along the osteoblastic lineage *in vitro*. These results suggest that LRP5 has a role during osteoblast proliferation and/or differentiation. When we measured lumbar spine bone mineral density in carrier and non-carrier relatives of OPPG patients we found that carriers had reduced bone mass compared to age- and gender-matched controls. These results suggest that bone mass accrual is sensitive to LRP5 quantity. We do not yet know the specific pathway by which LRP5 regulates bone mass accrual. However, published studies implicate two closely related family members (*Arrow* in Drosophila and *LRP6* in mice) in Wnt-mediated signal transduction, suggesting that LRP5 will also function in this signaling pathway.

Program Nr: 68 from the 2001 ASHG Annual Meeting

Disruption of a brain-to-skull LIM-homeobox link in patients with syndromic short stature and a germline mutation in *LHX4*. *K. Machinis[1], J. Pantel[1], I. Netchine[1], J. Léger[2], O.J.A. Camand[3], M-L. Sobrier[1], F. Dastot-Le Moal[1], P. Duquesnoy[1], M. Abitbol[3], P. Czernichow[2], S. Amselem[1]*. 1) Institut National de la Santé et de la Recherche Médicale (INSERM) U-468 and Service de Biochimie, Hôpital Henri Mondor, 94010 Créteil, France; 2) Service d'Endocrinologie et de Diabétologie, Hôpital Robert Debré Paris, France; 3) CERTO, Faculté Necker, Paris, France.

How the human brain develops while dictating morphogenesis and remodeling of the fit skull is a fundamental issue. Studies of genetically-engineered flies and mice revealed the role of orthologs of the human LIM-homeobox LHX4 — the molecular targets of which being so far unknown— in the control of neuron identity assignment and pituitary development. Although the targeted disruption of *Lhx4*is asymptomatic in mice in the heterozygous and lethal in the homozygous state, we considered *LHX4*as a candidate gene to be investigated in patients with pituitary defects. After having isolated and mapped *LHX4*, we report a family with a *LHX4*germline mutation which results in a disease phenotype revealed by a short stature, and combining forebrain (pituitary) and hindbrain (cerebellum) defects with abnormalities of the central skull base. This intronic mutation, which segregates in a dominant and fully penetrant manner over three generations, abolishes normal LHX4 splicing and activates two exonic cryptic splice sites, thereby predicting two different proteins deleted in their homeodomain sequences. We used two candidate-gene approaches to identify the targets of LHX4 which would account for the complexity of the disease phenotype. We showed that, unlike the mutant proteins, normal LHX4 enhances the transcription of both a pituitary and a bone turnover marker, which we unexpectedly found expressed in the pituitary. These findings, which unravel the molecular basis of a complex Mendelian disorder, point up the existence of a LIM homeobox-dependent protein network integrating brain- and bone-specific signaling pathways. They show how a single factor exerts fundamental pleiotropic effects throughout head morphogenesis by tightly coordinating brain development and skull shaping.

Program Nr: 69 from the 2001 ASHG Annual Meeting

Mutations in the vWFA domain of matrilin-3 cause multiple epiphyseal dysplasia. *M.D. Briggs[1], G.R. Mortier[2], K. Chapman[3], J. Loughlin[3], M.E. Grant[1], K.L. Chapman[1]*. 1) Wellcome Trust Centre for Cell Matrix Research, School of Biological Sciences, University of Manchester, England; 2) Department of Medical Genetics, Ghent University Hospital, Ghent, Belgium; 3) University of Oxford, Institute of Molecular Medicine, Oxford, England.

Multiple epiphyseal dysplasia (MED) is a relatively mild and clinically variable osteochondrodysplasia, primarily characterised by delayed and irregular ossification of the epiphyses and early onset osteoarthritis. Mutations in the genes encoding cartilage oligomeric matrix protein (COMP) and type IX collagen (COL9A2 and COL9A3) have been shown to cause different forms of MED. These dominant forms of MED (EDM1-3) appear to be restricted to mutations in the genes encoding structural proteins of the cartilage extracellular matrix, which have subsequently been shown to interact with high-affinity in vitro.

By performing a genome-wide screen in a four generation family exhibiting autosomal dominant MED, not linked to the EDM1-3 genes, we obtained significant genetic evidence for a novel MED locus on the short arm of chromosome 2 (2p24-p23). A search for candidate genes identified MATN3 within the critical region. Matrilin-3 is an oligomeric protein, which is present in the cartilage extracellular matrix (ECM). We identified two different missense mutations (V194D and R121W), in the exon encoding the vWFA domain of matrilin-3, in two unrelated families with MED. The valine at residue 194 and the arginine at residue 121 are conserved across all members of the matrilin family of proteins and also in different species. This observation suggests that these residues have major roles in the structure and/or function of the vWFA domain and the substitution of these residues, particularly by a hydrophobic residue such as tryptophan, will have a major effect on the folding of this domain.

These are the first mutations to be identified in any of the genes encoding the matrilin family of proteins and confirm an important role for matrilin-3 in the development and homeostasis of cartilage and bone.

.

Program Nr: 70 from the 2001 ASHG Annual Meeting

The first matrix metalloproteinase disease: MMP-2 deficiency results in a multicentric osteolysis syndrome. *J.A. Martignetti[1,2], A. Al Aqeel[3,4], W. Al Sewairi[3], C.E. Boumah[4], M. Kambouris[4,5], S. Al Mayouf[4], K.V. Sheth[4], O. Dowling[1], J. Harris[1], M.J. Glucksman[6], S. Bahabri[4], B.F. Meyer[4], R.J. Desnick[1,2].* 1) Dept of Human Genetics, Mount Sinai School of Medicine, New York, NY; 2) Dept of Pediatrics, MSSM, New York, NY; 3) Riyadh Armed Forces Hospital, Riyadh, Kingdom of Saudi Arabia; 4) King Faisal Specialist Hospital & Research Centre, Riyadh, Kingdom of Saudi Arabia; 5) Yale Univ. School of Medicine, New Haven, Connecticut; 6) Department of Neurobiology, MSSM, New York, NY.

The inherited osteolyses or vanishing bone syndromes are a group of rare disorders of unknown etiology characterized by the destruction and resorption of affected bones. Among these, the multicentric osteolyses are notable for interphalangeal joint erosions that mimic severe juvenile rheumatoid arthritis. We recently described an autosomal recessive form of multicentric osteolysis with carpal and tarsal resorption, crippling arthritic changes, marked osteoporosis, palmar and plantar subcutaneous nodules, and distinctive facies in a number of consanguineous Saudi Arabian families. We localized the disease gene to 16q12-21 using members of these families for a genome-wide search for microsatellite markers homozygous-by-descent. Haplotype analysis narrowed the critical region to a 1.2 cM region containing the MMP-2 (gelatinase A, collagenase type IV, EC 3.4.24.24) gene. No MMP-2 enzymatic activity was detected in the serum and/or fibroblasts from affected family members. Two family-specific homoallelic MMP-2 gene mutations (R101H and Y244X) were identified. The nonsense mutation deleted the substrate binding and catalytic sites and the fibronectin type II-like, and hemopexin/TIMP-2 binding domains. Based on molecular modeling, the missense mutation would disrupt hydrogen bond formation within the highly conserved prodomain adjacent to the catalytic zinc ion. Therefore, our results suggest that MMP-2 deficiency causes a multicentric osteolytic/arthritic syndrome, the first hereditary matrix metalloproteinase disease, and provide insight into the etiology of osteolysis and arthritis.

Program Nr: 71 from the 2001 ASHG Annual Meeting

Mutations in CD2BP1 that disrupt PTP PEST binding cause the allelic disorders familial recurrent arthritis and PAPA syndrome. *C.A. Wise[1], J.D. Gillum[1], C.E. Seidman[2], R. Veile[3], S. Bashiardes[3], M. Lovett[3]*. 1) Seay Research Center, Texas Scottish Rite Hospital for Children, Dallas, TX; 2) Department of Genetics, Harvard Medical School, Boston, MA; 3) Department of Genetics, Washington University School of Medicine, St. Louis, MO.

PAPA (pyogenic arthritis, pyoderma gangrenosum, and acne) syndrome, and familial recurrent arthritis (FRA), are inherited autoimmune disorders presenting as recurrent episodes of destructive, early-onset, pauciarticular sterile arthritis, ulcerative skin lesions and pre-adolescent, severe cystic acne. We recently localized FRA and PAPA syndrome to chromosome 15q by linkage and suggested that the disorders are allelic. We have now confirmed this by the identification of disease-causing mutations in the CD2 binding protein 1 (CD2BP1) gene. E250Q and A230T amino acid substitutions were identified in affected members of FRA and PAPA syndrome families, respectively. CD2BP1 or its murine ortholog PSTPIP1 are adaptor proteins known to interact with PEST-type protein tyrosine phosphatases as well as CD2, c-Abl, and Wiskott-Aldrich syndrome protein (WASP). The mutations we identified occur in a region of CD2BP1 potentially containing the PTP-PEST binding domain. In yeast two hybrid assays we have confirmed this interaction and demonstrate severe reduction of detectable binding for the CD2BP1 E250Q mutant protein. Our results support the conclusion that FRA and PAPA are allelic disorders, which we now refer to collectively as PAPA syndrome, and demonstrate the biological relevance of the E250Q mutation. Previous evidence supports the integral role of CD2BP1 and its interacting proteins in actin reorganization during cytoskeletal-mediated events. We suggest that CD2BP1 functions in a biochemical pathway(s) critical to the formation of the so-called T cell "immunological synapse". Alteration of this pathway(s) by heterozygous loss of CD2BP1/PTP PEST association or by WASP mutation confers the severe autoimmune inflammatory disease found in PAPA syndrome or immunodeficiencies found in WAS, respectively. Further investigation will delineate the pathogenesis of these and potentially other related disorders.

Program Nr: 72 from the 2001 ASHG Annual Meeting

Nail patella syndrome: A study of 123 patients from 43 British patients and the detection of 16 novel mutations of LMX1B. *E. Sweeney[1], A.E. Fryer[1], R.C. Mountford[2], A.J. Green[3], I. McIntosh[4].* 1) Merseyside and Cheshire Clinical Genetics Service, Royal Liverpool Children's Hospital, UK; 2) Merseyside and Cheshire Molecular Genetics Laboratory, Liverpool Women's Hospital, UK; 3) National Centre for Medical Genetics, Dublin, Ireland; 4) Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

Nail Patella Syndrome (NPS) is a very variable autosomal dominant condition affecting the nails, skeletal system, kidneys and eyes. Skeletal features include absent or small patellae, elbow abnormalities, talipes and iliac horns on x-ray. Kidney involvement may lead to renal failure and there is also a risk of glaucoma. NPS is caused by mutations in the transcription factor LMX1B at 9q34. LMX1B is involved in dorso-ventral patterning of the developing limb, regulation of collagen expression in the glomerular basement membrane, development of the anterior chamber of the eye, differentiation of dopaminergic neurons and also axonal migration. We present the results of a British study and suggest that gastrointestinal and neurological symptoms may also be part of the NPS phenotype. We also present the first data on the incidence of glaucoma in NPS. 98% had fingernail changes. 75% of patellae were hypoplastic and 9% absent. There was loss of extension in 70% of elbows and 12% of patients had pterygia. 19% had congenital talipes. 68% had iliac horns on X-ray. Renal involvement was present in 25% (33% in > age 40) - average age at detection 22 years (1-51). 2% had developed renal failure. 7% had ocular hypertension and 10% had glaucoma (12% and 17% respectively in > age 40) - average age at detection 48 years (23-78). Involvement of the GI tract was suggested in 31% by irritable bowel syndrome or constipation. 25% described peripheral neurological symptoms and 7% had epilepsy. Mutations of LMX1B were detected in 27/33 families including 16 novel mutations. There were 4 nonsense, 11 missense, 8 potential splice site and 4 frameshift mutations, consistent with the hypothesis that NPS is the result of haploinsufficiency for LMX1B. The broader range of symptoms than classically reported for NPS is in agreement with the pattern of LMX1B expression.

Program Nr: 73 from the 2001 ASHG Annual Meeting

Generation of *fanca/fancc* double knock-out mouse model for functional studies in Fanconi Anemia. *M. Noll[1], K.P. Battaile[1], R.L. Bateman[1], T.P. Lax[1], C. Reifsteck[1], S. Olson[1], R.K. Rathbun[2], G. Bagby[2], A. D'Andrea[3], M. Grompe[1]*. 1) Dept Molec/Medical Gen, L103, Oregon Health Sci Univ, Portland, OR; 2) Veteran's Administration Hospital, Oregon Health Sci Univ, Portland, OR; 3) Harvard Medical Institute, Boston, MA.

Fanconi anemia (FA) is a rare autosomal recessive disorder, clinical features of which include developmental abnormalities, bone marrow failure and cancer. Cellular hypersensitivity to interstrand DNA cross-linking drugs is the hallmark of this disease. FA is genetically heterogeneous and at least seven different complementation groups exist. To date six FA genes have been cloned (*-a, -c, -f, -g, -e and -d2*), but the biochemical function(s) of these proteins remain unknown. To study the pathophysiology and treatment of FA, we have generated mice deficient in *fancc* and *fanca* genes. Here, we report the generation of the *fanca* knock-out mouse, with a deletion of exon 37 resulting in a frame shift and absent protein. We have also generated double mutant animals.Similar to *fancc*-/-, *fanca*-/- and double mutants were viable and no macroscopic developmental abnormalities of the limbs or other organs were detected. The oldest double mutants are now 1 year old and do not display any hematological abnormalities or tumors. Similar to *fancc*-/- animals, they showed mild germ cell loss and hypersensitivity to DNA cross-linking agents. Testicular weights of *fancc*-/- (55.8±4 mg), *-a*-/- (79.6± 3 mg) and *-a/c* double mutants (46.8± 3 mg) were significantly reduced in comparison to the wild-type mice (97.4± 4 mg). Next, we established primary ear fibroblast cultures from the three genotypes and control littermates. Treatment with DEB and MMC did not reveal increased chromosome breakage in *-a/c* double mutants when compared to either mutant alone. This was corroborated by two more assays:(i) BFU-E and CFU-GM formation from the BM in response to different doses of MMC; (ii) ability of the fibroblasts to form clones in the presence of different concentrations of MMC. We thus conclude that the FANCA and C proteins are epistatic consistent with their role in a multimeric FA protein complex with a single function related to DNA damage responses.

Program Nr: 74 from the 2001 ASHG Annual Meeting

The Fanconi Anaemia protein FANCD2 associates with damaged DNA *in vivo*. *W. Wang[1], S. Meyn[1, 2]*. 1) Genetics, Hospital for Sick Children, Toronto, ON, Canada; 2) Molecular and Medical Genetics, University of Toronto, Toronto, ON, Canada.

Fanconi anaemia is characterized by pancytopenia, malformations, cancer, genetic instability and mutagen sensitivity. Recently it was shown that the protein coded by the *FANCD2* gene forms discrete nuclear foci with BRCA1 following X-irradiation, suggesting a role for FANCD2 in DNA repair. Now, using laser micro-irradiation, we report that FANCD2 protein localizes to nuclear regions containing high concentrations of double strand breaks (DSBs).

DSBs were introduced into selected regions of nuclear chromatin by incubation of human fibroblasts with Budr and Hoechst 33258 followed by exposure to a microbeam of 390 nm laser light. Normal cells were studied along with cells from individuals with ataxia-telangiectasia, Fanconi anaemia, and Bloom syndrome. In normal fibroblasts, localization of FANCD2 to DSBs was observed within 45 minutes of DSB induction. At later times, DSB-associated foci of FANCD2 colocalized with foci of BRCA1 and Rad51, proteins known to be involved in genetic recombination. In contrast, few DSB-associated FANCD2 foci colocalized with foci of nibrin, a component of the Mre11 non-homologous end-joining DNA repair complex.

Localization of FANCD2 to DSBs was independent of *BLM* function but required a functional *FANCA gene*, which is involved in mono-ubiquitination of FANCD2. DSB-associated FANCD2 foci were unusually intense in two different *ATM-/-* cell lines, while the association of BRCA1 with DSBs was markedly diminished.

Our results provide the first direct demonstration that a Fanconi anaemia protein can associate with damaged DNA in vivo and suggest that this association is dependent on mono-ubiquitination of FANCD2. While FANCD2 normally associates with BRCA1 following induction of DSBs, association with BRCA1 is not necessary for FANCD2 to localize to DSBs. Localization of FANCD2 to DSBs is an unusual cellular response to DSBs, in that it is ATM-independent. Colocalization of the laser-induced FANCD2 foci with foci of BRCA1 and RAD51 support our hypothesis that FANCD2 plays a role in the repair of DSBs by homologous recombination.

Program Nr: 75 from the 2001 ASHG Annual Meeting

Novel therapeutic strategies in Marfan syndrome (MFS). *E.R. Neptune[1], D.P. Judge[1], F. Ramirez[3], L.Y. Sakai[2], H.C. Dietz[1]*. 1) HHMI and Inst Genetic Med, Johns Hopkins Univ SOM, Baltimore, MD; 2) Shriners Hospital, Portland, OR; 3) Mt Sinai Med Ctr, New York, NY.

MFS is caused by mutations in the extracellular microfibril constituent fibrillin-1. Pulmonary manifestations can cause significant morbidity and have traditionally been attributed to destructive emphysema. Using an engineered mouse model of MFS, we recently demonstrated primary failure of distal alveolar septation in the absence of inflammatory or destructive changes. These findings were temporally and spatially associated with an increase in TGFb activation and signaling, leading to the hypothesis that microfibrils normally bind the large latent complex (LLC) of TGFb and sequester it from cell surface activators. This model is now supported by our demonstration that LTBP1, an integral component of the LLC, binds directly to fibrillin-1. Histologic analysis of lung from patients with MFS revealed enlarged distal airspaces with a paucity of alveolar septae and the absence of tissue damage, documenting the relevance of these findings to the human condition. Furthermore, we have demonstrated pulmonary hypertension in infants with severe MFS, presumably due to failure of the angiogenesis and vascular arborization that normally attends alveologenesis. Our prior work demonstrated partial rescue of pulmonary septation in heterozygous (but not homozygous) targeted mice after postnatal delivery of TGFb neutralizing antibody, suggesting a causal relationship between excessive TGFb signaling and the lung phenotype. We now show that prenatal delivery of neutralizing antibody completely restores normal postnatal lung architecture in both mutant genotypes. These effects could be recapitulated by administration of triamcinolone or retinoic acid (RA), agents capable of antagonizing TGFb signaling. High-dose steroids worsened impairment of alveolar septation, but RA consistently improved the lung phenotype in a dose-dependent manner. Other manifestations of MFS, including AV valve dysgenesis, correlated with increased TGFb activation in the relevant tissues. These data suggest a broadly applicable pathogenetic mechanism in MFS that may be amenable to currently available treatment regimens.

Program Nr: 76 from the 2001 ASHG Annual Meeting

Lathosterol Oxidase Disruption: A New Inborn Error of Cholesterol Biosynthesis. *P.A. Krakowiak[1], C.A. Wassif[1], L. Kratz[2], D.A. Vied[1], R.I. Kelley[2], F.D. Porter[1].* 1) Unit on Molecular Dysmorphology, Heritable Disorders Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland; 2) Kennedy Krieger Institute, Baltimore, Maryland.

Lathosterol oxidase (lathosterol 5-desaturase, EC 1.3.3.2) converts lathosterol to 7-dehydrocholesterol in the cholesterol biosynthetic pathway. Human and mouse malformation syndromes resulting from inborn errors of post-squalene cholesterol biosynthesis include desmosterolosis, Greenberg skeletal dysplasia, CHILD syndrome, bare patches mouse (bpa), Conradi-Hünermann-Happle syndrome (CDPX2), tattered mouse (td), and Smith-Lemli-Opitz syndrome (SLOS). These dysmorphic syndromes have overlapping but distinct phenotypes. Mutation of lathosterol oxidase has not been associated with either a human disorder or a natural mouse mutant. We disrupted the mouse *Sc5d* gene in order to determine the developmental consequences of this inborn error of cholesterol biosynthesis, to compare this mouse model to our SLOS mouse model, and to gain insight into potential human syndromes due to mutations of this gene. Homozygous pups are stillborn; growth retarded and have multiple congenital abnormalities. Dysmporphic facial features include micrognathia and cleft palate (Robin sequence) and closed nostrils. Limb malformations include shortening of both fore and hind limbs, soft tissue syndactyly, forelimb postaxial polydactyly, and a duplication of the distal phalanx of the fourth digit. Ribs are gracile and bones are hypomineralized. Internal organs are grossly normal but the liver is enlarged. Consistent with the expected enzymatic deficiency, GC/MS analysis demonstrated markedly elevated serum and tissue lathosterol levels and decreased serum and tissue cholesterol levels. To begin to elucidate the etiology of the various malformations, future directions include biochemical and enzymatic analysis and *in situ* expression studies of signaling molecules of the brain and limb bud.

Program Nr: 77 from the 2001 ASHG Annual Meeting

Localization of a susceptibility gene for familial non-medullary thyroid carcinoma (NMTC) to 2q21. *G. Romeo[1], J.D. McKay[1], F. Lesueur[1], L. Jonard[1], A. Pastore[1], J. Williamson[2], L. Hoffman[2], J. Burgess[2], M. Papotti[3], D. Goldgar[1], F. Canzian[1], M. Schlumberger[4] and International NMTC Consortium.* 1) International Agency for Research on Cancer, Lyon, France; 2) Royal Hobart Hospital Hobart, Tasmania, Australia; 3) Dipartimento di Anatomia Patologica University of Turin, Italy; 4) Institut Gustave Roussy, Villejuif, France.

The familial form of NMTC (FNMTC) is a complex genetic disorder, characterized by multifocal neoplasia and a higher degree of aggressiveness than its sporadic counterpart. In a large Tasmanian pedigree (Tas1) with recurrence of the most common form of NMTC, Papillary Thyroid Carcinoma (PTC), an extensive genome wide scan revealed a common haplotype on chromosome 2q21 in seven out of the eight PTC patients. In order to verify the significance of the 2q21 locus, linkage analysis was performed in an independent sample set of 80 pedigrees yielding a multipoint HLOD of 3.07 (a=0.42), NPL=3.19, (p=0.001) at marker D2S2271. Stratification based on the presence of at least one case of the follicular variant of PTC (fvPTC), the phenotype observed in the Tas1 family, identified 17 such pedigrees which showed a maximal HLOD score of 4.17 (a=0.80), and an NPL=4.99 (p=0.00002) at markers AFMa272zg9 and D2S2271 respectively. LOH and cytogenetic studies have implicated 2q, and specifically 2q21, in NMTC and in other forms of cancer suggesting the existence of one or more tumour suppressor(s) in this region where several candidate genes exist. However preliminary analysis of 34 sporadic and 8 familial PTC tumours with 5 microsatellites did not detect any LOH in this region. The Pax8 gene, which has been found translocated in NMTC, is excluded by recombinations in different families as it is situated centromeric to marker D2S2265. In summary, our data provide evidence in favour of the existence of a susceptibility locus for FNMTC at 2q21. This locus appears particularly relevant, although not confined, to families with at least one case of the fvPTC. Identification of critical recombinations should further define the 2q21 region and facilitate the positional cloning of this predisposing gene.

Program Nr: 78 from the 2001 ASHG Annual Meeting

Mapping of the Birt-Hogg-Dubé syndrome gene to chromosome 17. *S.K. Khoo[1], M. Bradley[2,3], F.K. Wong[1], M. Hedblad[2], M. Nordenskjöld[3,4], B.T. Teh[1]*. 1) Laboratory of Cancer Genetics, Van Andel Research Institute, Grand Rapids, MI-49503, USA; 2) Department of Dermatology and Venereology, Karolinska Hospital, Karolinska Institutet, Stockholm, Sweden; 3) Department of Molecular Medicine, Karolinska Institutet, Stockholm, Sweden; 4) Department of Clinical Genetics, Karolinska Hospital, Karolinska Institutet, Stockholm, Sweden.

Birt-Hogg-Dubé syndrome (BHD[MIM 135150]) is an autosomal dominant neoplasia syndrome characterized mainly by benign skin tumors, and to a lesser extent, renal tumors and spontaneous pneumothorax. To date the locus of the *BHD* gene remains unknown. Therefore we performed a genomewide linkage analysis using 400 microsatellite markers on a large Swedish BHD family to detect the susceptibility locus of *BHD*. Evidence of linkage was identified on chromosome 17p12-q11.2, with a maximum LOD score of 3.58 for marker D17S1852. Further haplotype analysis defined a 35 cM candidate interval between the two flanking markers, D17S1791 and D17S798. This information can be used to identify individual at risk in BHD families. It will also facilitate the identification of the *BHD* gene, which will lead to the understanding of its molecular etiology as well as the pathogenesis of its wide spectrum of tumors.

Program Nr: 79 from the 2001 ASHG Annual Meeting

A deep intronic mutation in CDKN2A predisposes to disease in a subset of melanoma pedigrees. *M. Harland, S. Mistry, D.T. Bishop, J. Newton Bishop.* ICRF Genetic Epidemiology, St James's University Hospital, Leeds, UK.

Germline mutations of CDKN2A at 9p21 have been shown to predispose to disease in melanoma pedigrees worldwide. However, there remains a significant proportion of melanoma pedigrees, with evidence of linkage to 9p21, in which mutations in CDKN2A have not been detected. Investigation of other potential tumour supressor genes at 9p21 and the promotor of CDKN2A has been unable to explain genetic predisposition to melanoma in these pedigrees. Here we describe a causal mutation, deep in intron 2 of CDKN2A (IVS2-105 A/G), detected in six UK melanoma pedigrees. The mutation creates a false GT splice donor site 105 bases 5' of exon 3, and has been demonstrated to result in aberrant splicing of the mRNA. The presence of this deep intronic mutation in a relatively large number of UK melanoma pedigrees, indicates that it may account for a significant proportion of 9p21 linked melanoma pedigrees with no detectable mutations in the coding region of CDKN2A. In addition, the identification of one deep intronic mutation in CDKN2A indicates the possibility of the existence of other similar splicing mutations located elsewhere in the CDKN2A introns. This discovery is therefore likely to have an impact on mutation screening in melanoma families.

Program Nr: 80 from the 2001 ASHG Annual Meeting

Germline mutations in BMPR1A, a TGFBR-SMAD family member, cause a subset of juvenile polyposis syndrome and Bannayan-Riley-Ruvalcaba syndrome. *C. Eng[1], X.P. Zhou[1], H. Hampel[1], K. Kurose[1], M. Aldred[1], R. Houlston[2], L.A. Aaltonen[3], I.P.M. Tomlinson[4].* 1) Human Cancer Genetics, Ohio State Univ, Columbus; 2) ICR, Surrey, UK; 3) Medical Genetics, Univ Helsinki, Finland; 4) ICRF, London, UK.

Juvenile polyposis syndrome (JPS) is a dominant hamartomatous polyposis syndrome with colon cancer risk. It is a clinical diagnosis of exclusion, and can be confused with other inherited hamartoma syndromes, eg Bannayan-Riley-Ruvalcaba syndrome (BRR) or Cowden syndrome (CS). While germline mutations of PTEN cause 80% CS and 60% BRR, mutations of MADH4/SMAD4 have been described in variable JPS probands. The frequency of MADH4 mutations in American JPS is 35-50% but <5% in mainly European JPS. BMPR1A belongs to the TGFBR superfamily upstream of the SMAD pathway. Because of BMPR1As location in proximity to PTEN, no genetic heterogeneity for CS and a report that PTEN might be regulated by TGFB, we examined BMPR1A as 1 of several candidates close to PTEN (JMG 2000;37:641) in PTEN mutation negative CS/BRR. Of 8 BRR cases without PTEN mutation, 1, possibly 2 (12-25%), carried germline BMPR1A mutations. 1 was a non-conservative missense mutation (A338D) not found in 172 control chromosomes and the other, a predicted splice. Both mutation positive individuals had BRR but also mixed hamartomatous and adenomatous polyps. BMPR1A analysis is on-going in further BRR and CS. Because of these observations and a recent report that 4/4 JPS families carry BMPR1A mutations (Nat Genet 2001;28:184), European JPS probands comprising 19 familial and 11 isolated cases without MADH4 mutations were analyzed for BMPR1A mutations. 10 (33%) were found to have BMPR1A mutations, 8 of which would result in truncated receptor. The 2 missense alterations are non-conservative (C124R, C376Y) and not found in 100 control chromosomes. Among familial cases, 7 (37%) carried BMPR1A mutations; among sporadic cases, 3 (27%) had mutations. Thus, germline BMPR1A mutations cause a significant proportion of JPS and might define a certain subset of BRR cases with specific colonic phenotype. Other members in the BMP-SMAD pathway should be considered candidates for MADH4/BMPR1A mutation negative JPS and perhaps PTEN/BMPR1A negative CS/BRR.

Program Nr: 81 from the 2001 ASHG Annual Meeting

2% of Men with Early Onset Prostate Cancer Harbour Deleterious Germline Mutations in the *BRCA2* Gene .

R. Eeles[1,2], S. Edwards[1], Z. Kote-Jarai[1], R. Hamoudi[1], D. Dearnaley[1,2], A. Ardern-Jones[2], A. Murkin[2], The CRC/BPG Collaborators[4], D. Easton[3]. 1) Cancer Genetics Unit, Inst Cancer Research, Sutton, Surrey, England; 2) Royal Marsden NHS Trust Downs Road Sutton Surrey SM2 5NG UK; 3) CRC Genetic Epidemiology Unit, Strangeways Research Laboratories Worts Causeway Cambridge, CB1 8RN UK; 4) The CRC/BPG Familial Prostate Cancer Study Collaborators UK.

Models suggest that about 40% of early onset (diagnosed at <55 years) cases of prostate cancer are due to a genetic predisposition (Carter et al., 1992). Deleterious mutations in the *BRCA2* gene predispose to a high risk of breast and ovarian cancer in female carriers in families with multiple cases of breast cancer. Male carriers in such families have an increased risk of prostate cancer of approximately 7-fold (an absolute risk by age 74 of 14%. Within the CRC/BPG UK Prostate Cancer Study, a multicentre national study, blood DNA from 275 young onset cases with prostate cancer diagnosed at <57 years has been collected. We have developed a rapid, more sensitive method of mutation detection for analysis of the entire coding region of the *BRCA2* gene in 45 multiplexed fluorescently labelled PCR fragments, in a modified gel mix, run on the ABI377 (F-MD; Edwards et al., 2001). Using F-MD, we have analysed the coding region of the *BRCA2* gene in these 275 early onset prostate cancer cases. Six deleterious mutations have been detected (five frameshifts; 2558insA; 6710delACAA; 7084delAAAAG; 7772insA; 8525delC and one splice site mutation IVS18-1g>c). This is a germline mutation rate in *BRCA2* of 2% (95% CI 0.8% - 4.7%. This equates to a relative risk of prostate cancer of 30-fold at <60 years. Half the cases with germline mutations had a family history of cancer, but half did not.

These results have implications for genetic testing of early onset prostate cancer cases and screening of their female relatives. Funded by Cancer Research Campaign, Prostate Cancer Charitable Trust, and Marion Silcock Legacy.

References Carter BS et al. (1992) Proc. Natl. Acad. Sci. USA, 89 3367-3371. Edwards S, Kote-Jarai Z, Hamoudi R & Eeles R (2001) Hum Mut, 17(3):220-232.

Program Nr: 82 from the 2001 ASHG Annual Meeting

A founder mutation in *MSH2* in the Ashkenazim. *W.D. Foulkes[1,8], I. Thiffault[1], D. Farber[1], S.B. Gruber[2], L. Tomsho[2], G. Rennert[3], M. Horwitz[4], T. Walsh[4], M.-C. King[4], N. Ellis[5], K. Offit[5], B. Bressac-de Paillerets[6], S. Grandjouan[6], J. Weitzel[7], F. Fujimura[7], P.H. Gordon[1], E. MacNamara[1], V. Marcus[8], G. Chong[1]*. 1) SMBD-Jewish General Hosp., McGill Univ., Montreal, Canada; 2) Univ. Michigan, Ann Arbor, MI; 3) CHS Cancer Control Center, Haifa, Israel; 4) Univ. Washington, Seattle, WA; 5) MSKCC, NYC, NY; 6) Institut Gustave Roussy, Paris, France; 7) City of Hope Cancer Center, Duarte, CA; 8) Montreal General Hosp., Montreal, Canada.

We identified a missense mutation, nt1906 G>C, resulting in A636P in MSH2 in an Ashkenazi Jewish (AJ) HNPCC family meeting Amsterdam Criteria 1(AC1) in 1997. Subsequently, 9 other AJ families have been found to carry this mutation. Segregation of the 10-marker haplotype (shared in all 7 families tested) to affected relatives was seen 3 times and 7/8 tumors tested were MSI-H. Where IHC for MSH2 was available, it showed absence of the protein in colorectal cancers (CRCs). Analysis of somatic cell hybrids carrying single alleles of *MSH2* indicated that the linked allele does carry the C nucleotide at 1906. Cancers in the 10 families are broadly HNPCC-related and 7/10 families are AC1 or 2 +. Notably, 3 families contained individuals with transitional cell ca & 2 had women with ovarian ca.

In 34 AJ CRC families from MSKCC, the A636P mutation was observed in 6; 5 of which met AC. In an incident series of 515 CRCs in Israel, we identified 3 (0.6%) mutation carriers. Cases with a family history of CRC or endometrial ca were more likely to carry the mutation ($p = .01$). Of 16 MSI-H CRCs so far identified in this series, 1 carries the A636P mutation. The mutation was not seen in 122 Sephardic Jews with CRC, in 379 Israeli AJ controls or in 271 US AJ *BRCA1/2* wt br. ca probands with a FH of CRC or ov.ca. Using the crystal structure of MutS, changing alanine 555 to proline interferes with the carbonyl group of neighboring phenylalanine and this change probably affects ATP binding or protein/protein interactions (pers. comm. W. Yang, NIH). While this pathogenic mutation is not common in the AJ population, it appears to be highly penetrant and to account for a significant fraction of AJ HNPCC.

Program Nr: 83 from the 2001 ASHG Annual Meeting

Polymorphisms and HNPCC: PMS2-MLH1 protein interactions diminished by 'single nucleotide polymorphisms'. *B. Gottlieb[1], Z. Qiang Yuan[1], L.K. Beitel[1], N. Wong[1,2], P.H. Gordon[1], Q. Wang[3], A. Puiseux[3], W.D. Foulkes[1,2], M. Trifiro[1]*. 1) Lady Davis Institute for Medical Research, Sir Mortimer B. Davis-Jewish General Hospital, Montreal, PQ, Canada; 2) Department of Human Genetics, McGill University, Montreal, PQ, Canada; 3) Department d'Oncologie Fondamentale et Appliquee, Centre Leon Berard, Lyon, France.

Hereditary nonpolyposis colorectal cancer (HNPCC) is one of the most common autosomal dominant inherited diseases. Mutations in the human mismatch repair proteins MLH1, MLH3, MSH2, MSH6, PMS1 and PMS2 have been found to co-segregate with HNPCC. The MLH1 and MSH2 proteins have been demonstrated to interact with PMS1, PMS2, and MSH6 proteins. A previous study reported that missense mutations in specific regions of *MLH1* can lead to defects in protein-protein interactions with *PMS2*. Here we report that three missense alterations previously identified as single nucleotide polymorphisms (SNPs) in *PMS2* (P511K, T597S and M622I) cause defective protein-protein interactions with hMLH1, even though the alterations are not in the previously reported interaction domain. These results suggest that other domains in *PMS2* affect MLH1-PMS2 interaction. This study also demonstrates that SNPs can result in gene alterations that indeed have a functional effect on protein phenotype. Thus, these three SNPs may ultimately represent variants with an increased risk factor for tumorgenesis in HNPCC. This study is one of the first to use a functional assay to appraise the role of SNPs and suggests that traditional definitions of polymorphisms and mutations are in need of reconsideration.

Program Nr: 84 from the 2001 ASHG Annual Meeting

***MSH2* in contrast to *MLH1* and *MSH6* is frequently inactivated by genomic rearrangements in Hereditary Non-Polyposis Colorectal Cancer.** *F. Charbonnier[1], S. Olschwang[2], Q. Wang[3], C. Boisson[2], C. Martin[1], M-P. Buisine[4], A. Puisieux[3], T. Frebourg[1].* 1) Genetics, INSERM EMI 9906, Faculty of Medicine, Rouen, FRANCE; 2) Fondation Jean Dausset-CEPH, Paris; 3) Oncologie Moléculaire, INSERM U453, Centre Léon Bérard, Lyon; 4) Biochimie et Biologie Moléculaire, CHU de Lille.

Genomic rearrangements of the mismatch repair (MMR) genes have been reported in several families with hereditary non-polyposis colorectal cancer (HNPCC), but the frequency of these alterations has not been estimated so far. Using the fluorescent multiplex PCR method, that we recently developed, we have therefore analyzed *MSH2, MLH1* and *MSH6* in 61 HNPCC families, without detectable point mutations of *MSH2* or *MLH1*, and corresponding to 49 families fulfilling the Amsterdam criteria for HNPCC and to 12 families selected for the presence of cases with multiple primary cancers belonging to the HNPCC spectrum. We detected 11 distinct genomic rearrangements of *MSH2* in 14 families (23%), whereas we found no rearrangement of *MLH1* and *MSH6*. Analysis of 31 other families partially meeting Amsterdam criteria revealed no additional rearrangement of *MSH2*. The majority of the rearrangements of *MSH2*, that we detected (9 /11), involved the region between the 5' end of the gene and intron 8, a region particularly rich in *Alu* repeats, and the 5' boundaries of 6/11 rearrangements were located within the 5' end of the gene, which density in *Alu* repeats, is extremely high. Therefore the distribution of the rearrangements of *MSH2* was consistent with the involvement of *Alu*-mediated recombination events. In HNPCC families without detectable *MSH2* or *MLH1* point mutations, the frequency of *MSH2* genomic rearrangements is therefore probably higher than that of point mutations within the other MMR genes. Furthermore, considering the frequency of *MSH2* genomic rearrangements, the simplicity and rapidity of their detection using fluorescent multiplex PCR, screening for *MSH2* rearrangements could be considered as the first step of the molecular analysis in HNPCC families.

Program Nr: 85 from the 2001 ASHG Annual Meeting

PTEN mutational spectrum in hereditary non-polyposis colon cancer syndrome-related endometrial carcinomas are distinct from that of sporadic microsatellite-unstable tumors. *X.P. Zhou, S. Kuismanen, P. Peltomaki, C. Eng.*
Human Cancer Genetics Program, Ohio State University, Columbus, OH.

Germline PTEN mutations cause Cowden syndrome which has an increased risk of breast, thyroid and endometrial cancers. Somatic genetic and epigenetic PTEN inactivation is involved in as high as 93% of sporadic endometrial carcinoma (EC) irrespective of microsatellite status. Hereditary non-polyposis colon cancer syndrome (HNPCC) is characterized by germline mutations in the mismatch repair genes and by microsatellite instability (MSI) in component tumors. EC is the most frequent extra-colonic cancer in HNPCC. We sought to determine if and how PTEN is involved in HNPCC-EC. 42 ECs belonging to 29 MLH1 or MSH2 mutation positive families were subjected to PTEN expression and mutation analysis. Immunohistochemical analysis with anti-PTEN antibody revealed 64% (27/42) of HNPCC ECs had absent or weak PTEN expression. The remaining 29% (12/42) had normal expression and 7% (3/42) mixed populations showing weak/absent as well as normal expression. Mutation analysis of the entire PTEN gene by DGGE and sequencing of 20 cancers with low or no PTEN expression revealed that 17 (85%) harbored somatic PTEN mutations. All 17 mutations were frameshift, 11 (65%) of which involved the poly-A tract in exons 7 or 8. Interestingly, 8/11 (72%) ECs with PTEN frameshift mutations also had loss of expression of MLH1, MSH2 and MSH6 while only 1/3 (33%, $P<0.05$) without frameshift mutation showed similar loss of expression. These observations suggest that somatic PTEN mutation, especially frameshift, is a consequence of profound mismatch repair deficiency in HNPCC-related EC. In contrast, among a series of 20 MSI+ sporadic ECs with somatic PTEN mutations, 10 were frameshift, of which only 1 (5%) affected the poly-A tract in exon 7. In summary, PTEN plays a prominent role in both HNPCC and sporadic ECs. However, the mutational spectra are distinct between sporadic MSI+ ECs and HNPCC-related ones. Thus, it is possible that different mechanisms exist in inactivating PTEN in endometrial carcinomas of HNPCC families and in sporadic MSI+ endometrial cancers.

Program Nr: 86 from the 2001 ASHG Annual Meeting

A systematic screen of the human genome for homozygous deletions in cancer. *P.A. Futreal, C.J. Cox, G.R. Bignell, H.R. Molloy, P.J. Stephens, R. Wooster, M.R. Stratton.* Cancer Genome Project, Sanger Ctr, Hinxton Cambs, England.

We have used high-density genotyping analysis (400 polymorphic micro-satellite markers giving an average 7.5Mb resolution) to identify regions of loss of heterozygosity (LOH) in over 665 cell lines. Having assigned LOH status (provisionally called if five contiguous markers are shown to be homozygous) to each chromosomal arm of a cell line, trays of 44 cell lines showing LOH on a given chromosome were prepared. Chromosomes 1-12 and 17 were separated into p and q arms with the remaining chromosomes screened in whole. These trays were then screened using a set of 5,800 Genethon genetically mapped markers (giving an approximate resolution of 0.5Mb), with the aim of trying to detect homozygous deletions (HD). As this is by far the largest screen for HD ever performed, we have used a high throughput means of analysis, which utilises the fact that the Genethon markers target CA repeats. By incorporating a third Taqman oligonucleotide in the reaction mix, specific for the CA repeat, we were able to routinely perform and analyse in excess of 30,000 PCRs daily. This high-density screen revealed the presence of 58 HD spread throughout the genome. Several of the HD map to known tumour suppressor genes (TSG) including PTEN, RB1, SAMD and p16, and we also detected two known fragile sites (FHIT and FRA 16D). Of the 52 HD not associated with known TSG or fragile sites, 8 were detected in more than one cell line, with at least 5 having different boundaries. More detailed mapping of these HD with a denser set of markers, again targeting CA repeats, revealed that while 4 HD are less than 1kb in size the rest vary between 35kb and 8.0Mb (larger deletions tend to be in less gene dense regions). Each HD on average contains 6-7 known genes. We are currently in the process of designing mutation detection primers for the genes in these regions and aim to screen all available cell lines showing LOH in that region.

Program Nr: 87 from the 2001 ASHG Annual Meeting

Essential vs Complex Autism: Definition of fundamental prognostic subtypes. *J.H. Miles, N. Takahashi, P. Sahota, C. Jones, R.E. Hillman.* Univ Missouri, Columbia, MO.

Heterogeneity within the autism diagnosis obscures the genetic basis of the disorder and impedes our ability to develop subgroup specific treatments. We report that by using 3 readily available tests, autism can be divided into two subgroups, essential autism and complex autism, with different outcomes & recurrence risks. Essential autism individuals are defined as non-dysmorphic, not microcephalic and having a normal brain MRI. From 1995-2001, 260 individuals met DSM-IV criteria for autistic disorder. All had a dysmorphology exam; 62% had a brain MRI, 56% an EEG. 5% (14/260) were microcephalic, 15% (39/260) dysmorphic, 35% (52/148) had an abnormal EEG & 27% (44/161) had an abnormal MRI. Individually, each feature predicted poorer outcomes. Together they defined a subgroup termed COMPLEX autism, comprising 30% (78/260) of the population with lower IQs ($P<0.01$), more seizures ($P<0.01$), more epileptiform EEGs ($P<0.005$) and lower heritability. All individuals with identifiable syndromes were in the complex group. The remainder have ESSENTIAL autism. Comparing the 83 individuals with essential autism who had MRIs with the complex group revealed improved outcomes [higher IQs ($P<0.01$) and fewer seizures ($P<0.01$)], greater sib recurrence (4.5% vs 1.1%), autism family history (24% vs 11%), higher male to female ratio (6.5:1 vs 3.6:1), and greater loss of language onset (44% vs 28%, $P<0.05$). Analysis of features relative to poor outcome at 10 yr (IQ <55, functionally non-verbal) revealed that microcephaly was most specific (100%) but only 15% sensitive, dysmorphology was 81% specific; 28% sensitive, epileptiform EEGs were 79% specific; 18% sensitive, MRI abnormalities were 46% specific; 38% sensitive. The 4 tests combined yielded 48% specificity and 59% sensitivity. Separating essential from complex autism should be the first diagnostic step as it leads to better prognostication, counseling and treatment. Linkage /sib pair analyses are expected to vary by subgroup. Since outcome is different, treatment research should distinguish between these groups. It is anticipated that each of the 4 features will be refined for more sensitive and specific separation.

Program Nr: 88 from the 2001 ASHG Annual Meeting

Refinement of a 400 kb critical region allows genotypic differentiation between isolated lissencephaly, Miller-Dieker syndrome and other phenotypes secondary to deletions of 17p13.3. *C. Cardoso[1], R.J. Leventer[1,2], H.L. Ward[1], J. Chung[1], A. Gross[1], A. Leung[1], C.M. Lese[1], D.T. Pilz[3], A.H. Olney[4], O.M. Mutchinick[5], W.B. Dobyns[1,2], D.H. Ledbetter[1]*. 1) Departments of Human Genetics and; 2) Neurology, University of Chicago, Chicago, IL; 3) Institute for Medical Genetics, University Hospital of Wales, United Kingdom,; 4) Center for Human Genetics, University of Nebraska Medical Center, Omaha, NE,; 5) Departamento de Genetica, Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico.

Deletions of 17p13.3 including the LIS1 gene result in the brain malformation lissencephaly characterized by reduced gyration and increased cortical thickening, however the phenotype can vary from isolated lissencephaly sequence (ILS) to Miller-Dieker syndrome (MDS). At the clinical level, these two phenotypes can be differentiated by the presence of significant dysmorphic facial features and a more severe grade of lissencephaly in MDS. Previous work suggested that children with MDS have a larger deletion than those with ILS, yet the precise boundaries of the MDS critical region and causative genes other than LIS1 have never been fully determined. We have completed a physical and transcriptional map of the 17p13.3 region from LIS1 to the telomere. Using fluorescence in situ hybridization, we have mapped the deletion size in 14 children with ILS, 12 children with MDS and 4 children with 17p13.3 deletions not involving LIS1. We show that the critical region that differentiates ILS from MDS at the molecular level can be reduced to 400 kb. Using somatic cell hybrids from selected patients we have identified, by PCR, 7 genes that are exclusively deleted in patients classified as having MDS. In addition, deletion of the genes CRK and 14-3-3 epsilon delineates patients with the most severe lissencephaly grade. Based on existing functional data for these genes, we suggest that deletion of 14-3-3 epsilon in combination with a deletion of LIS1 account for the complete agyria seen only in patients with MDS. We conclude that 14-3-3 epsilon may also play a role in cortical development.

Program Nr: 89 from the 2001 ASHG Annual Meeting

LIS1 missense mutations cause milder lissencephaly phenotypes including a child with normal intelligence. *W.B. Dobyns[1,2], R.J. Leventer[1,2], K. Swanson-Petras[1], A. Weiss[1], D.T. Pilz[3], N. Matsumoto[4], C.M. Lese[1], C. Cardoso[1], D.H. Ledbetter[1]*. 1) Departments of Human Genetics and; 2) Neurology, University of Chicago, Chicago, IL; 3) University Hospital of Wales, Cardiff, UK; 4) Nagasaki University School of Medicine, Nagasaki, Japan.

Classical lissencephaly is a neuronal migration disorder resulting in a thickened cortex and reduced gyration usually causing severe mental retardation, intractable epilepsy and cerebral palsy. The majority of patients have mutations or deletions of the LIS1 gene on 17p13.3 or mutations of the DCX gene on Xq22.3. Most patients with lissencephaly secondary to LIS1 mutations or deletions have a severe malformation consisting of generalized agyria and pachygyria with greatest severity in posterior brain regions. Increasing experience now suggests that the phenotypic spectrum in patients with LIS1 mutations is wider than previously thought. We have screened 219 children with classical or variant lissencephaly or the related malformation subcortical band heterotopia for abnormalities of the LIS1 or DCX genes. We found 65 LIS1 deletions, 33 LIS1 mutations and 59 DCX mutations. Of those with LIS1 mutations, only 5 had a missense mutation. Here, we describe the clinical and imaging features and mutation data of the 5 patients with missense mutations of LIS1 and emphasize one child with normal intelligence. We conclude that patients with missense mutations of LIS1 have a wider and milder spectrum of cortical malformations and clinical sequelae compared to patients with other mutation types. The severity of the phenotype is consistent with the predicted effect of the specific amino acid substitution on LIS1 protein function. We suggest that the few patients found thus far with missense mutations of LIS1 results from an under-ascertainment of patients with more subtle malformations and that abnormalities of the LIS1 gene may account for a greater spectrum of neurological and developmental problems in childhood than has previously been appreciated.

Program Nr: 90 from the 2001 ASHG Annual Meeting

1p deletion syndrome: a common, important and often missed cause of developmental delay/mental retardation.

A. Battaglia[1], D.H. Viskochil[2], S.O. Lewin[2], M. Bamshad[2], Z. Chen[2], A.N. Lamb[3], J.P. Palumbos[2], L. Berkheim[2], J.C. Carey[2]. 1) Inst Child Neurology & Psych, Stella Maris nst/Univ Pisa, Pisa, Italy; 2) Div Medical Genetics, Dept Pediatrics/Univ Utah, SLC, UT, USA; 3) Genzyme Genetics, Santa Fe, NM, USA.

We report on 4 patients ranging in age, at the first observation, from 1 month to 4 1/2 years, whose karyotype showed monosomy 1p36.3. The diagnosis was made in 3 of them by high resolution banding (550-700 band level), whereas subtelomere FISH analysis (1pSUBTEL probe, p58 and D1Z2 Oncor probes) was necessary in the other one. Two of the patients were karyotyped respectively at age 1 and 4 months, because of multiple congenital anomalies; while the other 2 were studied (at age 3 1/2 and 4 1/2 years respectively) as part of a neurologic work-up for developmental delay/mental retardation (DD/MR). On examination, OFC was at/below the 2%, height and weight ranged between <3% and 25%. Tower skull, prominent forehead, deep-set eyes, mid-face hypoplasia, broad nasal root and bridge, ear anomalies of variable degree, long philtrum, high-arched palate with thick alveolar ridges, small wide-spaced teeth, relative prognathism, bilateral brachydactyly with camptodactyly and abnormal dermatoglyphics, and short feet were seen in all patients. Additional findings in some of the patients include heart defects, brain anomalies, hearing loss, optic nerve coloboma, pyloric stenosis and cecum malposition. Developmental delay/mental retardation was present in all, and seizures were observed in 2. From recent literature reports it looks like 1p36.3 deletions account for just under 1% of idiopathic MR/DD. In view of the commonness of this condition and the non-specificity of its features, we would suggest performing subtelomere FISH analysis for 1p36 in all patients with apparent idiopathic MR/DD.

Program Nr: 91 from the 2001 ASHG Annual Meeting

Population data suggest deletions of 1p36 are common. *H.A. Heilstedt, B.C. Ballif, L.A. Howard, L.G. Shaffer.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Deletion of the most telomeric band on chromosome 1 (1p36) results in a clinically recognizable syndrome of characteristic facial features, seizures, mental retardation, hearing loss and growth failure. We have collected a total of 59 patients with deletions of 1p36 and have analyzed the deletion sizes and parental origins for each. We found that there is not a consistent sized deletion but rather variability in the molecular size and thus compliment of genes missing from each patient. Parental origin analysis reveals that 68% of deletions are from the maternally-derived chromosome. There does not appear to be a difference in phenotype based solely on parental origin. However, the larger sized deletions are generally from the paternally-derived chromosome. About 18% of patients show derivative chromosomes. However, most are de novo with only ~ 6% of patients inheriting an unbalanced segregant from a parental translocation. This frequency of parental rearrangements is somewhat lower than other telomeric deletion syndromes such as Wolf-Hirschhorn, Cri du chat or Miller-Dieker, but still important for recurrence risk assessment and counseling. The ethnic backgrounds of our patients are diverse and include Caucasian, African-American and Hispanic origins. Originally, the prevalence was estimated to be 1 in 10,000. However, based on the fact that almost half (40%) of our patients have had a normal chromosome analysis in the past, this incidence is probably an underestimate. We predict the true incidence to be closer to 1 in 5,000, making it the most common terminal deletion in the population.

Program Nr: 92 from the 2001 ASHG Annual Meeting

Partial trisomy 1q: A recognizable phenotype in five individuals. *L. Hudgins[1], D. Sternen[2], C. Dolan[2], C. Disteche[2].* 1) Pediatrics/Med. Gen., Stanford Univ, Stanford, CA; 2) Univ. of Washington, Seattle, WA.

Partial trisomy of the long arm of chromosome 1 is rare and is usually associated with monosomy of another autosome, which complicates the phenotype. There are only a few case reports of partial trisomy 1q in the literature. We report the first series of patients with partial trisomy 1q and describe a recognizable phenotype.

We describe 5 patients with partial trisomy 1q involving the region 1q32 to 1qter. Three have an X;1 translocation and 2 have a duplication of the long arm of one chromosome 1. The oldest patient is in her early 20's. All have growth retardation, which in most cases was of prenatal onset. All have severe to profound mental retardation. None are able to ambulate unassisted and none have verbal language. Major malformations are surprisingly rare in this group of patients. Three of the 5 have cardiac malformations, 2 with ventricular septal defects and 1 with an atrial septal defect. Two males have genital abnormalities including mild hypospadias, micropenis, and undescended testes. One patient has hydrocephalus requiring shunting, 1 has metopic craniosynostosis, 1 has a horseshoe kidney, and 1 had congenital cataracts requiring removal of the lenses. Minor anomalies are common and consistent from patient to patient. Four of the 5 have dysplastic ears which are described as lowset with cupping on one side and overfolded superior helix on the other. Four of the 5 also have micrognathia. Plagiocephaly and a long philtrum are also common features. Medical complications include seizures in 3 of the 5, and chronic aspiration in 3. Chronic otitis media and/or serous otitis media were also seen in 3 patients.

In general, the prognosis for patients with partial trisomy 1q is poor from a developmental standpoint. However, because of the relative lack of serious malformations and serious medical complications, these patients may reach early adulthood.

.

Program Nr: 93 from the 2001 ASHG Annual Meeting

Genotype/Phenotype Analysis of 5 families with small deletions in the WS region. *C.A. Morris[1], C.B. Mervis[2], M.T. Keating[3], A.D. Stock[1], P.A. Spallone[1], B.P. Klein-Tasman[4], B.F. Robinson[5].* 1) Dept Pediatrics, Genetics Div, Univ Nevada Sch Medicine, Las Vegas, NV; 2) University of Louisville, Louisville, KY; 3) Harvard University, Boston, MA; 4) University of Wisconsin, Milwaukee, WI; 5) Georgia State University, Atlanta, GA.

Supravalvar aortic stenosis is an autosomal dominant elastin arteriopathy caused by mutation in the *elastin* gene. Williams syndrome is a sporadic condition with a microdeletion that encompasses *ELN*. The classic Williams syndrome deletion is approximately 1.5 Mb. In the course of study of SVAS families, 5/14 kindreds have been identified with deletions in the WS region. The deletions range in size from 83 kb to approximately 800 kb. The smallest deletion involves only two genes in the region, *ELN* and *LIMK-1*. The other four kindreds have a deletion in common that includes *ELN*, *LIMK-1*, and *RFC2*. The deletions in the five families span the WS deletion region. A total of 21/44 individuals are affected in the 5 families. Investigations include dysmorphology examination, cardiology examination including echocardiography, detailed psychometric evaluation, and molecular and cytogenetic analysis of the WS region of chromosome 7. The affected family members in these kindreds have SVAS, the WS cognitive profile, and some of the WS facial features (none have a classic WS facial gestalt). No affected individuals have mental retardation or hypercalcemia. Only one of the 5 families has affected individuals with the WS personality. None of the unaffected individuals have any manifestations of the WS phenotype. Families with small deletions in the WS region are important in the study of genotype/phenotype correlations.

Program Nr: 94 from the 2001 ASHG Annual Meeting

Transient Neonatal Diabetes: the 6q24 phenotype. *I.K. Temple[1], D.J.G. Mackay[2], A.-M. Coupe[2], H. Cave[3], M. Polak[3], R. Siebert[4], J.C.K. Barber[2], D.O. Robinson[2], J.P.H. Shield[5]*. 1) Wessex Clinical Genetics Service, Southampton University Hospital Trust, Southampton, UK; 2) Wessex Regional Genetics Laboratory, Salisbury, Wiltshire, UK; 3) Laboratoire de Biochimie Génétique and Service d'Endocrinologie, Hôpital Robert Debré, Paris, France; 4) Department of Human Genetics, University of Kiel, Kiel, Germany; 5) Department of Child Health, Royal Hospital for Sick Children, Bristol, UK.

Transient Neonatal Diabetes (TND) is a rare type of diabetes, which presents soon after birth, resolves by eighteen months and predisposes to diabetes later in life. We have ascertained 34 TND patients with an abnormality of chromosome 6.

The patients could be classified into three aetiological groups: Group 1 had paternal UPD6; Group 2 had a duplication of 6q24 which was paternal in origin (where tested) and Group 3 consisted of patients with a loss of methylation at a CpG island within the TND critical region.

Analysis of the clinical findings in this cohort has allowed us to define the 6q24 TND phenotype accurately. The major findings included growth retardation at birth, presentation within a week of life and recovery by 12 weeks. At presentation ketonuria was the exception and insulin levels were low in the presence of high blood sugar levels. Islet cell antibodies were absent. Macroglossia and umbilical herniae were the only dysmorphic features consistently reported. Normal growth and development after the initial diabetic period was the norm but many relapsed in later childhood. No significant differences were found between aetiological groups.

Three relatives of group 2 probands gave a history of type 2 diabetes. Duplication of 6q24(pat) was identified in 2/3 so far tested, thereby extending the 6q24 phenotype to include an adult onset presentation with no early history.

Program Nr: 95 from the 2001 ASHG Annual Meeting

Deletion 22q13 syndrome - under-recognized and under-diagnosed. *M.C. Phelan[1], R.C. Rogers[2], D.B. Everman[2], G.A. Stapleton[2], N.R. Powers[3], S.R. Shaw[3].* 1) T C Thompson Children's Hosp, Chattanooga, TN; 2) Greenwood Genetic Center, Greenwood, SC; 3) The Children's Hospital, Greenville, SC.

Deletion of 22q13 is generally considered a rare cytogenetic finding. However, with advances in FISH technology and the availability of subtelomeric probes, over 70 cases of deletion 22q13 have been described. We recently compared the features of 37 individuals with deletion 22q13 to 24 cases from the literature (Phelan et al, Am J Med Genet, 2001). We now report data from 33 additional individuals and 8 new cases from the literature. The most common features of deletion 22q13 are hypotonia, global developmental delay, normal to accelerated growth, and absent or delayed speech. Minor dysmorphic features include dysplastic toenails, relatively large fleshy hands, prominent and/or dysplastic ears, pointed chin, dolicocephaly, ptosis, and epicanthal folds. Other features include lack of perspiration, hearing loss, and seizures. Autistic behaviors have been reported in several cases from the literature, yet only 1 of 20 patients in our series who were tested for true autism met the strict diagnostic criteria. A number of individuals from our series and from the literature were initially diagnosed as atypical Angelman syndrome; others were detected fortuitously when FISH studies performed for VCF revealed deletion of the control probe at 22q13. Because the major features of this deletion syndrome are nonspecific and the deletion itself so subtle, deletion 22q13 remains a diagnostic dilemma and is currently under-diagnosed. High resolution chromosome analysis and FISH with a probe specific for 22q13 are indicated in any hypotonic newborn in whom a specific diagnosis is not apparent. Likewise, these studies should be considered in older individuals with a history of hypotonia, absent or delayed speech, global developmental delay, and normal growth. Even in the absence of one of the four major features, other minor dysmorphic features may suggest this diagnosis. The number of reported cases of deletion 22q13 syndrome continues to increase. Once appropriate studies are performed on at risk individuals, deletion 22q13 is likely to be one of the most common deletion syndromes in man.

Program Nr: 96 from the 2001 ASHG Annual Meeting

What environmental and interventional differences may contribute to the outcome in individuals with distal 5p deletions. *M.E. Carlin[1,2], D.R. Campbell[2,3], H-R. Schulte[4].* 1) Gen & Dev Ctr of SW,Ft.Worth,TX; 2) The 5p-Society:Family Support Group (USA); 3) Auburn U, AL; 4) German 5p- Family Support Group.

Data from a questionnaire and/or examination of >200 individuals from N.Amer/USA (NAUSA) with *Cri-du-Chat* syndrome and other distal 5p deletions documenting the incidences of the phenotypic features and various abnormalities has already been presented/published. This ongoing study now includes data on >500 individuals from diverse geographical areas: NAUSA, S.Amer.,Australia, Scandinavia, Germany, Italy, Belgium/Netherlands, and the British Isles/Ireland. While the nature and incidences of the physical features have been comparable across nationalities, some other interesting differences have been noted. In addition to antibiotics, chronic otitis is treated with drainage tubes twice as frequently in the USA as in Europe. Conversely, salivary duct surgery, rarely performed in the USA, is frequently used(35%-50%) in Europe to decrease drooling. Some of the more common congenital orthopedic anamolies, often treated with braces, orthotics and orthopedic surgery in the USA and Australia, are more often successfully treated with intensive exercises and graded therapies, especially in Germany. Indeed, the average age for independent walking is 12-18 mos earlier in the British Isles. Similar differences are also present in the cognitive/language spheres. Though sign language is introduced universally at 12-18 mos, those in NAUSA add and use signs at a dimished rate, possibly due to the complexities of American Sign Language; whereas those Europeans, who utilize simpler systems(MaKaTon),consistently use and more rapidly add signs. Additionally, most Europeans (esp.Germans) >10 yrs have some verbal speech, whereas <50% of comparable English-speaking individuals do. Moreover, many nations outside USA offer a variety of other services: weekly-monthly respite care; compensation for disabilities, including monies for environmental adaptation; equestrian therapy; generous health insurance benefits; and various community-based employment opportunities. Further outcomes research should be able to document which interventions yield the greatest gains in each circumstance.

Program Nr: 97 from the 2001 ASHG Annual Meeting

A dominant *FTL* mutation causes a novel and pleiotropic basal ganglia disorder that can mimic torsion-dystonia, Huntington's Disease and parkinsonism. *C. Fey[1], A.R.J. Curtis[1], P.F. Chinnery[2], C.M. Morris[3], P.G. Ince[4], A. Coulthard[2], M.J. Jackson[2], W.A. Barker[1], A. Curtis[1], J. Burn[1]*. 1) Institute of Human Genetics, University of Newcastle, Newcastle-upon-Tyne, UK; 2) Department of Neurology & Radiology, NHS Trust, Newcastle-upon-Tyne, UK; 3) Joint MRC-Newcastle University Centre for Development in Clinical Brain Ageing, Newcastle-upon-Tyne, UK; 4) Department of Pathology, University of Sheffield, Sheffield, UK.

A pedigree from the north west of England contains multiple individuals affected by dominant, fully penetrant, late-onset degeneration of the basal ganglia, variably presenting with extrapyramidal features similar to dystonia, Huntington's Disease or parkinsonism. Symptoms include choreoathetosis, spasticity and rigidity, sometimes showing acute progression but with no significant dementia. Cavitation of the basal ganglia occurs at a late stage. The disorder was mapped by linkage analysis to a 3.5 cM region of 19q13.3, and candidate genes were chosen on the basis of potential involvement in neurodegeneration. We describe a frameshift mutation in the ferritin light chain gene (*FTL*) predicted to alter 22 carboxy-terminal residues. Patients show abnormal aggregates of ferritin and iron in the brain and low serum-ferritin levels. The same mutation was found in unrelated subjects with isolated dystonia that were negative for the *DYT1*-mutation and some with extrapyramidal features similar to the main familiy. Ferritin comprises 24 subunits of two types forming soluble, hollow sphere and has two main functions: controlled storage and detoxification of iron. Brain iron deposition increases normally with age and is a suspected causative factor in several neurodegenerative diseases, possibly by its involvement in toxic free radical reactions. An abnormality in ferritin strongly implicates a primary role for iron in the pathogenesis of this new disease, for which we propose the name neuroferritinopathy.

Program Nr: 98 from the 2001 ASHG Annual Meeting

A novel pantothenate kinase gene (PANK2) is defective in Hallervorden-Spatz syndrome. *B. Zhou[1], S.K. Westaway[2], B. Levinson[1], M.A. Johnson[2], J. Gitschier[1], S.J. Hayflick[2].* 1) Howard Hughes Medical Institute and Dept. of Medicine and Pediatrics, University of California, San Francisco, CA; 2) Dept. of Molecular and Medical Genetics, Pediatrics and Neurology, Oregon Health Sciences University, Portland, OR.

Hallervorden-Spatz syndrome (HSS) is an autosomal recessive, progressive neurodegeneration disorder. This extrapyramidal disease includes a variety of symptoms and difference in age of onset. The cardinal feature of HSS is pathological iron deposition in the globus pallidus and unique brain MRI findings ("eye of the tiger"). Genetic mapping indicates that most classical HSS is due to mutations in a single locus at 20p13. Linkage analysis data from about 100 independent families refined the HSS region to a definite region of 3 cM and a very likely region of 1.5 cM. Systematic positional cloning effort of HSS was undertaken within the 3 cM region. Out of about 20 genes examined within this locus, a novel but highly conserved pantothenate kinase homologue (here designated as PANK2) was found to be mutated in most classical HSS patients. Surprisingly, many atypical HSS patients also carry mutations in this gene. These mutations, which include nonsense, frameshift and missense changes, are mostly unique, consistent with our linkage studies that showed no founder effect. We demonstrated that PANK2 is able to complement E. coli pank mutation in vivo, indicating PANK2 is truly a pantothenate kinase. Pantothenate kinase catalyses the first step during CoA synthesis and subsequently cysteine was incorporated into the phosphorylated pantothenate. Interestingly cysteine accumulation has been found in the globus pallidus of HSS patients. We propose that the presence of cysteine aggravates the oxidative stress in this already iron-rich tissue. An animal model, a drosophila pank mutant (fumble), displays movement uncoordination, similar to that observed in human HSS patients. Our findings have strong implications for the mechanism of HSS including iron deposition in the brain, and open a completely new pathway for the study of other neurodegeneration diseases.

Program Nr: 99 from the 2001 ASHG Annual Meeting

Identification and characterisation of the gene for Chorea-acanthocytosis. *L. Rampoldi[1], C. Dobson-Stone[1], J.P. Rubio[2], A. Danek[3], A.H. Németh[1], A.P. Monaco[1].* 1) The Wellcome Trust Centre for Human Genetics, Oxford, U.K; 2) The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia; 3) Neurologische Klinik, LMU, Munich, Germany.

Chorea-acanthocytosis (CHAC, MIM 200150) is a rare autosomal recessive neurodegenerative disorder characterised by the gradual onset of hyperkinetic movements (chorea), and abnormal, spiky erythrocyte morphology (acanthocytosis), which occurs at variable levels in affected individuals. Clinical findings include a progressively debilitating dysphagia, motor or vocal tics, dystonia, parkinsonism, epileptic seizures and sensory-motor axonal neuropathy. The clinical and neuropathological findings closely resemble those observed in Huntington's disease. We identified a novel, ubiquitously expressed gene, in the CHAC critical region on chromosome 9q21 and screened for mutations in the 11 CHAC families previously reported in the linkage study. We found 3 nonsense, 9 frameshift, 1 splice-site and 3 missense mutations in Chorea-acanthocytosis patients. Additional mutations have been identified in a second set of CHAC patients. The Chorea-acanthocytosis gene, which we named *CHAC*, is organised in 73 exons and encodes a 3174 amino acid protein that is evolutionarily conserved showing sequence similarity with putative *Drosophila* and *C. elegans* proteins and the vacuolar protein sorting-associated protein 13 (VPS13) of *S. cerevisiae* and *S. pombe*. This suggests a role for CHAC in protein sorting from the Trans Golgi Network (TGN) to late endosomes, lysosomes and the plasma membrane as well as the involvement of this process in neurodegeneration. We are conducting a preliminary study to characterise the function of the CHAC protein using markers for several organelles (TGN, early, late and recycling endosomes, lysosomes, plasma membrane) and antibodies for sorted proteins in patient and control lymphoblasts and fibroblasts. Fusion protein contructs are being prepared to obtain an anti-CHAC antibody, which will be used in immunofluorescence and immunoprecipitation experiments.

Program Nr: 100 from the 2001 ASHG Annual Meeting

Modeling the X-linked dominant deletion syndrome microphthalmia with linear skin defects (MLS) in the mouse indicates that deletion of the *HCCS* gene causes the male lethality of MLS. *I.B. Van den Veyver, T.A. Cormier, S.K. Prakash, B. Xu, A. McCall, H.Y. Zoghbi.* Baylor Col Medicine, Houston, TX.

MLS is an X-linked dominant male-lethal deletion syndrome characterized by skin defects of the face and neck, microphthalmia, sclerocornea, corpus callosum agenesis and other brain anomalies. We identified three genes in the 450Kb critical region on Xp22.3: *MID1* (telomeric), *HCCS* (middle) and *ARHGAP6* (centromeric). Because no MLS patients without deletions are known, we performed mutation analysis of patients with other X-linked dominant conditions that have phenotypic overlap with MLS (Aicardi and Goltz syndromes) to find which of the three genes may cause the MLS phenotype. We found no mutations. We then generated lines of mice targeted with two *Lox*P sites to create three overlapping deletions of the murine MLS-homologous region. All three deletions inactivate *Hccs*, which encodes a holocytochrome *c*-type synthetase. This protein incorporates cytochrome *c* in the mitochondrial respiratory chain, and is essential for its normal function. Our attempts to generate mice null for this gene led to death of the male (XY) ES cells. Consistent with this, we could not generate deletions at the ES-cell stage, which also suggests that Hccs is essential for survival. When targeted mice were bred with a line that expresses Cre recombinase at the zygote and early embryo stages, we saw only mosaic patterns of deletions in females, but never heterozygous, homozygous or hemizygous deleted mice. Deleted mice appear to die prior to E9.5. To rescue this lethality, we crossed double-targeted mice to mice expressing a human BAC with the entire *HCCS* gene. This yielded viable homozygous and hemizygous deleted mice. Our data indicate that deletions containing *Hccs* are more severe in mice than in humans, since no heterozygote deleted mice survived without the *HCCS* transgene. We conclude that loss of HCCS causes the male lethality of MLS syndrome. We are now determining the exact timing and cause of embryonic death and are generating tissue-specific deletions, targeted to tissues affected in human MLS syndrome. Our studies should elucidate the role of mitochondrial function in CNS and eye development.

Program Nr: 101 from the 2001 ASHG Annual Meeting

Mutations in *SLC19A3* encoding a novel transporter cause biotin-responsive basal ganglia disease. *W. Zeng[1], E. Al-Yamani[2], J.S. Acierno[1], P. Ozand[2], J.F. Gusella[1]*. 1) Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown, MA and Department of Genetics, Harvard Medical School; 2) Department of Pediatrics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia.

Biotin-responsive basal ganglia disease (BBGD) is a autosomal recessive disorder characterized as early onset of a subacute encephalopathy with confusion, dysarthria and dysphagia, then progress to severe cogwheel rigidity, dystonia and quadriparesis. These symptoms disappear within 1 month if high dosage biotin (5-10mg/kg/day) is administered and reappear if biotin is discontinued. Using linkage analysis, we have mapped the BBDG gene to chromosome 2 and defined a candidate interval of ~2 Mb between markers *D2S2354* and *D2S1256* in 2q36.3. Computer analysis identified a putative novel gene sharing homology with folate and thiamine transporters (*SLC19A1, SLC19A2*) proteins, which has recently been named *SLC19A3*. *SLC19A3* is comprised of 6 exons, encoding a protein of 496 aa containing 12 putative transmembrane domains. The cDNA sequence consisted of 1660 nt that includes a predicted 1488 bp ORF. Northern analysis showed the presence of 3.5, 2.6, and 2.0-kb transcripts in many tissues, with greatest abundance in placenta, kidney and liver. Mutation screening was performed by SSCP analysis and direct sequencing in four BBDG families representing two different chromosomal haplotypes. Two missense mutations were found in exon 2 and 5, changing amino acids highly conserved in similar transporters in man, mouse and lower organisms. The *SLC19A3* alterations segregated with the disorder in each BBGD family and were not detected on 364 control chromosomes. These findings indicate that *SLC19A3* is likely to be a biotin transporter whose normal activity is crucial to basal ganglia function.

Program Nr: 102 from the 2001 ASHG Annual Meeting

Mutations in a novel GTPase cause autosomal dominant hereditary spastic paraplegia. *X. Zhao[1], D. Alvarado[1], S. Rainier[1], R. Lemons[1], P. Hedera[1], C. Weber[1], T. Tukel[2], M. Apak[2], T. Heiman-Patterson[3], L. Ming[1], M. Bui[1], J.K. Fink[1,4]*. 1) Dept Neurology, Univ Michigan, Ann Arbor, MI; 2) University of Istanbul, Institute of Child Health, Division of Medical Genetics, Istanbul, Turkey; 3) Hahneman University School of Medicine Department of Neurology; 4) Geriatric Research Education Clinical Center, Ann Arbor Veterans Affairs Medical Center.

Autosomal dominant hereditary spastic paraplegia (HSP) is a genetically heterogeneous group of disorders characterized by insidiously progressive lower extremity spastic weakness. Autosomal dominant HSP linked to the SPG3 locus on chromosome 14q11-21 begins in childhood and is often severe. We recently reduced this locus to a 2.7 cM interval between D14S259 and D14S978. Through analysis of candidate genes in BAC contigs spanning this locus we now report discovery of disease-specific missense mutations in a novel gene (designated atlastin) in affected subjects from three unrelated HSP SPG3-linked kindreds. Atlastin encodes a novel protein of 558 amino acids that is highly homologous to human guanylate binding protein 1 (hGBP1), a member of the dynamin family of large GTPases. Disease-specific atlastin mutations (missense) occur in the globular region containing the GTPase domain. Northern blot and RT-PCR experiments indicate that atlastin is expressed predominantly in the central nervous system. Dynamins play essential roles in diverse processes including synaptic vesicle recycling, internalization of ligand activated receptor complexes, post-golgi vesicle trafficking, and maintenance and distribution of mitochondria. Identification of atlastin gene mutations in SPG3-linked HSP and evidence that atlastin is a dynamin family member will permit direct investigation of the molecular basis for progressive distal axonal degeneration in this form of HSP and related neurodegenerative disorders.

Program Nr: 103 from the 2001 ASHG Annual Meeting

Mice lacking paraplegin, a mitochondrial AAA protease involved in hereditary spastic paraplegia, show axonal degeneration and abnormal mitochondria. *F. Ferreirinha[1], A. Quattrini[2], V. Valsecchi[1], A. Errico[1], A. Ballabio[1], E.I. Rugarli[1]*. 1) TIGEM, Naples, NA, Italy; 2) Dipartimento di Neuroscienze, Unita' di Neuropatologia, Ospedale San Raffaele, Milano, Italy.

Hereditary spastic paraplegia (HSP) is a progressive neurological disorder characterized by degeneration of the corticospinal tracts. Paraplegin, the gene responsible for the autosomal recessive form linked to chromosome 16q (SPG7), encodes a protein homologous to yeast mitochondrial metalloproteases. Consistently, paraplegin was found to localize to mitochondria and typical signs of OXPHOS defects were observed in muscle biopsies from patients with paraplegin deficiency. In order to study the pathogenesis of HSP due to lack of paraplegin, we have generated a mouse model by inactivation of the Spg7 gene. Paraplegin-deficient mice are born at the expected mendelian ratio, are viable, and fertile. Homozygous -/- animals display an impaired performance on the rotarod apparatus, starting at 6 months of age and worsening with age. Semithin sections of the spinal cord of 7-months-old mice show axonal swellings that are more prominent in the lateral columns of the lumbar spinal cord, consistent with a retrograde axonopathy. This phenotype is slowly progressive, with signs of axonal degeneration becoming prominent at 12 months of age. Mice older than one year also show axonal swelling and degeneration in the optic and sciatic nerves, and muscle abnormalities. Electron microscopy demonstrates that swollen axons contain accumulated organelles and neurofilaments, suggesting that axonal transport is impaired. Axons are filled with abnormal mitochondria, such as gigantic mitochondria, mitochondria with disrupted cristae, and vacuole-containing mitochondria. These alterations preceded axonal pathology, suggesting that axonal enlargement and degeneration are due to impaired mitochondrial function. The abnormal mitochondrial morphology and the homology between paraplegin and other molecules involved in membrane fusion and organelle biogenesis lead us to hypothesize that paraplegin might be involved in promoting efficient mitochondrial fusion and/or division.

Program Nr: 104 from the 2001 ASHG Annual Meeting

Early-onset ataxia with ocular motor apraxia and hypoalbuminemia, a variant form of Friedreich's ataxia, is caused by mutations in a novel HIT superfamily protein, aprataxin. *H. Date[1], O. Onodera[1], S. Igarashi[1], A. Yokoseki[1], H. Tanaka[1], R. Koike[1], S. Tsuji[1], K. Iwabuchi[2], H. Nagatomo[2], Y. Sekijima[3], T. Hiroi[4], K. Uekawa[5], E. Uyama[5], T. Yuasa[6], Y. Awaya[7], K. Saitou[8], T. Sakai[8], S. Sugano[9].* 1) Niigata University; 2) Kanagawa Rehabilitation Center; 3) Shinshu University School of Medicine; 4) Hosoki Hospital; 5) National Kumatomo Minami Hospital; 6) Kohnodai Hospital,National Center of Neurology and Psychiatry; 7) Tokyo Women's Medical University of Medicine; 8) National Chikugo Hospital; 9) University of Tokyo.

We have identified a unique group of patients whose clinical presentations are characterized by autosomal recessive inheritance, early age of onset, progressive ataxia, absent tendon reflexes, distal loss of position and vibration sense, pyramidal weakness, and hypoalbuminemia. Based on these clinical characteristics, we have collected 7 Japanese pedigrees. Despite the similarity in clinical presentations with those of Friedrich's ataxia (FA), expansion of GAA repeats was not present, and linkage to FA locus was also excluded. We have conducted genome-wide linkage analysis. Despite the effort, unequivocal conclusion has not been obtained. Very recently, Moreira et al. mapped a unique autosomal recessive ataxia,early-onset ataxia associated with ocular motor apraxia (AOA), to 9p13. Given some similarities in the clinical presentation with AOA, we confirmed that our families are also linked to the same locus. The highest pair-wise LOD score was 7.72 at D9S1845. Taking advantage of a strong linkage disequilibrium, we were able to narrow down the candidate region to a 350 kb segment, and eventually to identify the causative gene,aprataxin; that belongs to a histidine triad (HIT) superfamily. Mutations including deletion/insertion and missense mutations were identified in all the 7 families. Furthermore, we also identified the same mutation in aprataxin gene in 2 patients who had been previously reported as AOA, suggesting that both conditions share identical molecular defects. Here, we propose early-onset ataxia with ocular motor apraxia and hypoalbuminemia (EAOH) for this unique neurodegenerative disease.

Program Nr: 105 from the 2001 ASHG Annual Meeting

Mutations in the ganglioside-induced differentiation-associated protein 1 (GDAP1) gene cause axonal Charcot-Marie-Tooth disease. *F. Palau[1,2], A. Cuesta[1], L. Pedrola[1], T. Sevilla[3], J. Garcia-Planells[1], E. LeGuern[4], J.J. Vilchez[3].* 1) Laboratory of Genetics and Molecular Medicine, Instituto de Biomedicina, CSIC,Valencia, Spain; 2) Department of Genetics, University of Valencia, Valencia, Spain; 3) Department of Neurology, Hospital Universitari La Fe, Valencia, Spain; 4) INSERM U289, Hôpital de la Salpêtrière, Paris, France.

Charcot-Marie-Tooth (CMT) disease is the most common peripheral inherited neuropathy. Two main forms, demyelinating CMT1 and axonal CMT2, are described. Autosomal dominant, autosomal recessive, and X-linked Mendelian patterns have been reported. More than 20 loci and genes have been associated with CMT and related neuropathies.

We show genetic analysis and gene characterization of the responsible gene of a form of autosomal recessive CMT2 associated with vocal cord paresis. By linkage analysis and homozigosity mapping the disease locus was mapped between markers D8S551 and D8S541 in 8q21. A gene, *GDAP1*, mapped within the critical region and expressed in the nervous system was chosen as a candidate gene. Northern blot analysis of different human tissues revealed a major 3.9-kb transcript with the highest level of expression in whole brain and spinal cord. The gene sequence contains an ORF of 1077 bp and encodes a 358 aa protein. Protein has two glutathione S-transferase domains and at least one transmembrane domain in C-terminal. Mutation analysis of three families showed three different mutations: family LF38 was homozygous for Q163X mutation, family LF20 was heterozygous for Q163X and T228fsX290 mutations, and family LF249 was heterozygous for Q163X and S194X mutations. Abnormal expression of *GDAP1* gene may represent a new pathogenic mechanism causing peripheral neuropathy.

Program Nr: 106 from the 2001 ASHG Annual Meeting

Beta-synuclein gene alteration in Dementia with Lewy Bodies (DLB). *P. Limprasert[1], J.P. Taylor[2], J. Leverenz[3,4,5], D. Tsuang[4,5], L. Bonner[5], F. Tanaka[2], T.D. Bird[3], B.L. Sopher[1], A.R. La Spada[1].* 1) Dept Lab Medicine, Univ Washington, Seattle, WA; 2) NINDS, NIH, Bethesda, MD; 3) Dept Neurol, Univ Washington, Seattle, WA; 4) Ment Illn Res Ctr, Puget Sound VA, Seattle, WA; 5) Dept Psych & Behav Sci, Univ Washington, Seattle, WA.

DLB is characterized by dementia, fluctuating cognition, hallucination and parkinsonism. The diagnosis is confirmed at autopsy by documenting cortical and subcortical Lewy bodies. Although DLB shares both clinical and pathological features with Alzheimer's disease and Parkinson's disease, it appears to be a distinct disorder. We ascertained six multiplex families that show dominant inheritance of DLB. A number of observations suggested that the synuclein gene family (alpha, beta, gamma) deserved consideration as candidate genes for the disorder. To screen for mutations, we sequenced the synuclein coding regions in 6 index cases. We found a nucleotide alteration in codon 123 of the beta-synuclein gene from one case. This nucleotide substitution changed a proline to a histidine (P123H). We developed a PCR assay to test for the presence of the change and, using this assay on 830 chromosomes, we are yet to detect a single P123H alteration in individuals of the same ethnicity as the affected patient (American-Caucasian). Although the proband had an affected mother and sister (both deceased), DNA is not available from either. We compared the beta-synuclein coding sequence of closely related mammalian species and noted that proline is conserved in all sequenced species at this position. Furthermore, substitution of hydrophilic histidine for uncharged, sterically-constrained proline should disrupt the tertiary structure. As aggregates of beta-synuclein have been detected in DLB, we transfected HEK293T cells with beta-synuclein expression constructs and exposed cells to oxidative stress (10 mM FeCl2 for 27 hrs). Despite comparable expression levels, P123H-transfected cells formed cytoplasmic aggregates while cells transfected with wild type beta-synuclein did not. Based on these data, we propose that the P123H mutation in beta-synuclein is potentially responsible for the DLB phenotype in this family.

Program Nr: 107 from the 2001 ASHG Annual Meeting

A linkage disequilibrium map of chromosome 22. *L.R. Cardon[1], G.R. Abecasis[1], E. Dawson[2], S. Bumpstead[2], Y. Chen[2], S. Hunt[2], J. Pabiol[2], T. Dibling[2], E. Tinsley[2], S. Curby[2], D. Carter[2], M. Papaspyridonos[2], S. Livingstone[2], R. Ganske[3], K. Rice[2], P. Deloukas[2], I. Dunham[2], D. Bentley[2]*. 1) Wellcome Trust Ctr, Univ Oxford, Oxford, England; 2) The Sanger Center, Hinxton, Cambridge CB10 1SA, United Kingdom; 3) Third Wave Technologies, Madison, Wisconsin, 54719-1256, USA.

Allelic association studies of complex traits have met with limited success. Amongst the many reasons for this outcome is a lack of knowledge about background levels of linkage disequilibrium (LD) and a catalogue of common haplotypes in the human genome, which are necessary for marker selection in mapping projects and for critical evaluation of their findings. Characterization of genomic LD patterns is likely to assist in abroad range of asssociation applications, including positional cloning and candidate gene studies, pharmacogenetics, and genome-wide association screeening. We have constructed a full LD map of chromosome 22, comprising 1506 SNPs genotyped on 77 Utah/CEPH inidividuals from 10 families. The markers have an average spacing of ~22kb and have been interdigitated with the publicly available CEPH microsatellite markers for unambiguous phase assignment. A genotyping error rate of 0.5% was determined by duplicate genotyping of 8 individuals. Two extended regions of very high LD (average D' > .5 for markers separated by less than 500 kb) were detected, spanning several hundred kb (and >50 markers). These regions are significantly correlated with gene density (coding bp/kb), CpG representation and genetic distance. In contrast, several other regions were detected in which little LD was apparent, including one tract of ~6 MB (>200 markers) near the telomere of 22q. Application of a number of distributional models suggested that these patterns differed substantially from random variation. These findings demonstrate the LD can be characterised at a chromosome-wide scale and, together with a a catalogue of common conserved reference haplotypes, should greatly facilitate trait-based association studies.

Program Nr: 108 from the 2001 ASHG Annual Meeting

Linkage disequilibrium in 487 human genes. *T. Acharya, J.A. Schneider, R. Jiang, G.F. Vovis, J.C. Stephens.* Population Genomics, Genaissance Pharmaceuticals, New Haven, CT.

We discovered 5,948 SNPs in approximately 1.2 Mb of genomic DNA from 487 human genes. DNA sequencing was performed on 82 unrelated individuals including African-Americans, Asians, European-Americans and Hispanic Latinos. The data were collected from specific gene regions including exons, exon-intron boundaries, untranslated regions and 5' and 3' flanking regions. Haplotypes were inferred using an Expectation-Maximization algorithm, and linkage disequilibrium was calculated with the D' statistic between pairs of SNPs within each gene. A majority of the inter-SNP distances were less than 5 kb. The analyses focused on pairs of high-frequency SNPs (>10% in each ethnic group) that were usually present in all four ethnic groups. The average level of linkage disequilibrium was relatively high within all genes and all ethnic groups (D'>0.5); however, patterns varied substantially within individual genes. In addition, the level of linkage disequilibrium in most of the genes did not show a consistent decay with increasing physical distance. While this last observation may be influenced by the relatively short inter-SNP distances in our data, these results suggest that patterns of human linkage disequilibrium are complex and gene-dependent, and underscore the need to construct detailed, empirical linkage disequilibrium maps of the human genome.

Program Nr: 109 from the 2001 ASHG Annual Meeting

Sequence variation and linkage disequilibrium in a large set of human genes involved in inflammation. *M.A. Eberle[1], M. Rieder[2], D.A. Nickerson[2], L. Kruglyak[1,3].* 1) Division of Human Biology and Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, Washington; 2) University of Washington, Department of Molecular Biotechnology, Seattle, Washington; 3) Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, Washington.

As part of a Program for Genomic Applications (PGA), our group, known as SeattleSNPs, is discovering and analyzing the patterns of sequence variation in genes and pathways that underlie inflammatory responses in humans. In the discovery project, we are sequencing 47 individuals (24 of African-American descent and 23 of European descent). A sample of 47 individuals (94 chromosomes) should contain virtually every SNP with minor allele frequency of greater than 5% in the population. To date, we have sequenced 25 genes in their entirety, representing 211,708 bp of genomic sequence from each individual. We have identified 1032 SNPs, or approximately 1 SNP every 209 bases. We are currently discovering SNPs at a rate of ~400 a month. At present, the data set contains 1317 pairs of SNPs such that both SNPs lie in the same gene and have minor allele frequency greater than 20%. The distances between two SNPs in a pair cover the range from 0 to 15 kb. These pairs are useful for measuring the level and genomic extent of linkage disequilibrium. We will describe sequence diversity, SNP allele frequency distributions, and linkage disequilibrium in the African-American and European populations, as well as in the combined population. We have developed a computer program that uses the coalescent approach to simulate sequence samples of large chromosomal segments under different demographic scenarios. We will present the results of modeling our data set in order to make inferences regarding human evolutionary history.

Supported by the National Heart Lung and Blood Institute U01 HL66682 and U01 HL66642.

Program Nr: 110 from the 2001 ASHG Annual Meeting

Significant linkage disequilibrium (LD) cover wide intervals in alleles of the late settlement subpopulations of Finland. *T. Varilo[1], T. Paunio[1], A. Parker[3], J. Meyer[3], J.D. Terwilliger[4], L. Peltonen[1, 2]*. 1) Dept. of Molecular Medicine, NPHI, HKI, Finland; 2) Dept. Of Human Genetics, UCLA, LA, CA; 3) Millennium Pharmaceuticals Inc, Cambridge, MA; 4) Dept. of Psychiatry and Columbia Genome Center, Columbia U, NY.

LD has been used in the identification and fine mapping of disease genes in isolates. For multifactorial disease genes its usefulness has been seriously challenged. We compared the extent of LD in two populations from the well established genetic isolate of Finland: One sample representing late settlement area, inhabited 15 generations ago, and the other a regional subpopulation, Kuusamo, founded by 39 families 13 generations ago. We genotyped 53 microsatellite markers on 1q and 5q. The genealogy of the late settlement (n=95) and Kuusamo families (n=54) were defined delicately to the 1800s. The markers had an average marker interval of 0.77 cM. For 1q 22 markers over 27 cM and for 5q 31 markers over 14 cM were genotyped. Both samples revealed significant evidence for LD, with an explicit distance-correlated pattern. Significantly more LD was found in Kuusamo, than in the more heterogeneous population of late settlement. For chromosome 1q, in the Kuusamo sample, 77% of pairs showed significant LD over the interval less than 0.5 cM, 57% over 0.5-3,5 cM, 46% over 3,5-12 cM, and 9% over 12 cM intervals. For the late settlement alleles, the corresponding figures were 85%, 25%, 6% and 6%. For chromosome 5q, in Kuusamo, 59% of pairs expressed LD over the distance less than 0.5 cM, 48% over 0.5-2 cM, and 16% over 2 cM distances. For late settlement, the figures were 69%, 34% and 7%. Our findings of wide genetic regions revealing LD in the Finnish alleles evidence for the benefits of the well characterized genetic isolates also for the genome-wide searches of common predisposing alleles. Due to a small number of founders, affected individuals in isolated populations and especially in young regional subpopulations are related and share common predisposing alleles IBD. Our data emphasize the significance of genealogical information and selection of the samples when choosing study strategies (Peltonen et al 2000 Nat Rev Genet).

Program Nr: 111 from the 2001 ASHG Annual Meeting

The effect of ascertainment bias on estimates of linkage disequilibrium. *J.M. Akey[1], J. Wakeley[2], K. Zhang[1], M. Xiong[3], L. Jin[1]*. 1) Environmental Health, University of Cincinnati , Cincinnati , OH; 2) Organismic and Evolutionary Biology, Harvard University, Cambridge, MA; 3) Human Genetics Center, University of Texas-Houston, Houston, TX.

Linkage disequilibrium is becoming an integral tool in contemporary genetics research. Recently, a high-density single nucleotide polymorphism (SNP) marker map has been constructed and deposited into the public domain, which consists of approximately 1.65 million SNPs. However, the majority of these SNPs were identified by data-mining procedures that considered only a single population and a small number of chromosomes. Therefore, it is important to consider the effect of ascertainment bias (AB), which arises when SNPs are identified in one sample and subsequently genotyped in a second sample, on estimates and comparisons of LD between populations. To address this problem, we have performed extensive coalescent simulations that model two subpopulations where SNPs are "identified" in one population and then used to estimate D' (a measure of LD) in both populations. In our simulations, we consider how different demographic histories, migration rates, size of the region considered, and number of chromosomes used for SNP discovery affect the magnitude of AB. In general, we have found that AB can be a significant problem in estimating and comparing LD between populations and that the severity of AB is a complex function of the genetic divergence between populations, the size of the region considered, and the demographic history of the populations. AB can lead to errors of approximately 40% when comparing D' between two populations. However, a balanced SNP discovery strategy, where an equal number of chromosomes from both populations are used, significantly reduces the effect of AB. The consequences of AB in the context of LD mapping of genes underlying complex diseases will also be discussed. In conclusion, our preliminary results suggest that it is necessary to perform separate SNP discovery projects in each population studied in order to accurately compare the level of LD between populations and raises serious concerns as to the general utility of the currently available SNP resources.

Program Nr: 112 from the 2001 ASHG Annual Meeting

Linkage disequilibrium mapping for complex traits through haplotype/genotype sharing. *S. Zhang[1], Y. Li[1], X. Zhu[2], R.S. Cooper[2], H. Zhao[1]*. 1) Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT; 2) Department of Preventive Medicine and Epidemiology,Loyola University Medical Center, Maywood, IL.

Several statistical methods have been developed to jointly analyze multiple markers to identify mutations underlying complex traits. However, most of these methods rely upon explicit assumptions on the recombination history from common ancestors to the sampled individuals, the availability of haplotype data, and the trait being discrete. In this presentation, we will describe a novel nonparametric approach developed by us to relate phenotype similarity to haplotype/genotype similarity as a tool to localize the disease mutation. This approach does not depend on specific population genetics assumptions and is applicable to both qualitative traits and quantitative traits. The basic idea behind our approach is that the correlation between haplotype/genotype similarities and phenotype similarities is expected to be higher around the disease mutation location than that around other locations on the chromosome. Within this general approach, we have developed both haplotype-based methods and individual-based methods. For haplotype-based methods, two haplotype similarity measures have been considered: (1) the shared length of two haplotypes around the candidate locus; and (2) a weighted measure of halotype sharing according to the distance from the candidate locus. For individual-based methods, we correlate trait value similarities with their genotype similarities in two ways parallel to the two measures for haplotypes. We have evaluated and compared these methods through simulation studies and their applications to real data sets associated with angiotension I-convertng enzyme gene. The results show that the individual-based method with the weighted measure of genotype similarity was the best method among the four whereas the haplotype-based method with the shared length as a measure of haplotype similarity was the worst method. Overall, our results demonstrate that our methods performed better than existing methods to localize the mutation site and are robust to population history and disease model.

Program Nr: 113 from the 2001 ASHG Annual Meeting

COMPARISONS OF HAPLOTYPE BASED METHODS USING INTRAGENIC SNPs IN CANDIDATE GENES. *C. Bourgain, E. Genin, F. Clerget-Darpoux.* genetic epidemiology, INSERM U535, Kremlin-Bicetre, France.

The choice of an optimal marker strategy while analyzing intragenic SNPs is presently of crucial importance, given the increasing amount of available data. Classical case/ control association studies or family based association tests such as the TDT are very popular. However, as these methods are not able to analyze multiple markers simultaneously, different extensions have been proposed (Clayton and Jones, 1999; Toivonen et al 2000; Zhao et al 2000; Bourgain et al, 2000) in order to use multiple markers. In the present study, the efficiency of these 4 haplotypic methods to detect the role of candidate genes is evaluated and compared between them and with the TDT. Simulations of an intragenic SNP map in recently founded populations are performed assuming one or several of the SNPs are functional for different genetic models, and in particular for different modes of SNP combinations underlying the genetic susceptibility (epistasis or heterogeneity). If haplotypic methods perform better in situations of heterogeneity, the TDT remains the most powerful approach in epistasis models as long as the marginal effect of one the SNPs involved in the susceptibility remains important. Haplotypic methods perform better than the TDT when each marginal effect is small, particularly when functional SNPs are adjacent. Given the similar characteristics of intragenic LD in both old large populations and recently founded populations, in particular the weak correlation between LD and distance, our results are not likely to be specific to founder populations and can be generalized.

Program Nr: 114 from the 2001 ASHG Annual Meeting

Estimation of Haplotype Frequencies from Diploid Data. *G.R. Abecasis[1], R. Martin[2], S. Lewitzky[2]*. 1) Wellcome Trust Center for Human Genetics, University of Oxford, Oxford, England; 2) Human Genetics, Millenium Pharmaceuticals Inc., Cambridge, MA.

Extended haplotypes are likely to be more informative for complex trait mapping studies than single markers. However, currently available tools for haplotype estimation (such as Arlequin, Phase, Genehunter and Simwalk2) are limited in their handling of missing data, family structures, number of markers and disequilibrium patterns. Here, we describe a method for estimating haplotypes in family or case control data and companion computer program.

Our strategy allows for many markers, arbitrary family structures and missing data. We start by identifying all possible non-recombinant haplotypes in the region of interest and proceed to maximize their frequencies using an expectation maximization algorithm. Finally, we use estimated haplotype frequencies to identify the most likely haplotypes for each individual. When the number of markers under investigation is large, a divide-and-conquer algorithm is used to explore the space of possible haplotypes. We examined the properties and limitations of the method through simulation. Finally, as a practical application, we constructed a catalogue of common haplotypes among 1506 chromosome 22 SNPs genotyped in families and unrelated individuals.

Program Nr: 115 from the 2001 ASHG Annual Meeting

The absolute and relative accuracy of haplotype inferral methods and a consensus approach to haplotype inferral. *S. Orzack[1], D. Gusfield[2], V.P. Stanton, Jr.[3]*. 1) Fresh Pond Research Institute, Cambridge, MA; 2) Department of Computer Science University of California Davis, California 95616; 3) Variagenics, Inc. Cambridge, MA 02139.

Theoretical and practical considerations suggest that a more complete causal understanding of complex traits like many human diseases will be gained by haplotype analysis, since such traits may usually be the partial result of many genetic determinants. Several important algorithms for haplotype inferral have been developed but there has been little assessment of their performance with respect to data. We describe the results of our analyses of the accuracy of presently-available computer algorithms for random genotypic data for the ApoE locus in humans. There are 9 SNP sites and 80 individuals in our data set; all individuals have experimentally-inferred haplotypes. Algorithms studied include those based on the Expectation-Maximization approach and on the rule-based approach. We compared the frequency distributions of haplotypes predicted by the various algorithms. They differed significantly with regard to their predicted frequency distributions and also with regard to their success at predicting the list of real haplotypes. Accuracy was also assessed absolutely by comparing the identities of predicted haplotype pairs with the identities determined by direct molecular analysis. The algorithms differed significantly with regard to their success at predicting haplotype pairs; most predicted less than 80 percent of the haplotype pairs correctly. We conclude that present algorithms cannot serve by themselves as accurate haplotype predictors. To this extent, we describe consensus methods for dividing data sets into those genotypes in need of experimental inferral and those genotypes not in need of such inferral because their algorithmic inferral has a very high probability of being correct. In this way, one can hope to obtain correct identification of the haplotypes of all of the individuals in a sample. The consensus method is based upon a rule-based algorithm which can generate alternative haplotype pairs for a given individual. (Research supported by a National Science Foundation Award to D.G.).

Program Nr: 116 from the 2001 ASHG Annual Meeting

Determining success of haplotyping algorithms using densely mapped genomic regions. *D.V. Zaykin*[1], *M.G. Ehm*[1], *B.S. Weir*[2]. 1) Department of Population Genetics, GlaxoSmithKline Inc, Research Triangle Park, NC; 2) Program in Statistical Genetics, Department of Statistics, North Carolina State University, Raleigh, NC.

Recent research suggests that using statistically inferred haplotype frequencies may increase power of marker-disease association studies. Such conclusions are often made using simulated data where one must assume a population size, admixture model, specifics of recombination process, marker allele frequencies, etc. These parameters affect the balance between the stronger association provided by haplotypes and the loss of power due to increase in degrees of freedom and phase uncertainty. Thus, it is unclear whether adding additional markers to construct haplotypes will actually increase power. To address these concerns, we examined the power of haplotype and single-marker tests using 551 markers typed in a 12 cM region in 421 unrelated Caucasian individuals. The large number of markers allows us to compute empirical error rates and statistical power, as well as average optimal haplotype sizes using real marker data instead of simulated data. Our approach is to treat each marker, in turn, as an unobserved disease locus and examine strength of the correlation between the disease status influenced by that site and a nearby single marker as well as haplotypes. We investigate the error rate and power of the likelihood ratio test (LR) and the novel composite haplotype method (CHM). The LR test contrasts the expectation-maximization based likelihoods in cases, controls and the pooled sample. The CHM is a new method based on the extension of the composite linkage disequilibrium (Weir, 1996) to multi-allelic multiple markers. The CHM is applicable to discrete and continuous traits. An important feature of CHM is that it does not assume Hardy-Weinberg equilibrium (HWE) in affected and non-affected individuals. Instead, it uses deviations from HWE at the haplotype level to facilitate differences between these groups. We show that the CHM provides a more powerful test.

Program Nr: 117 from the 2001 ASHG Annual Meeting

Linkages associated with thrombocytopenia in pedigrees multiplex for lupus are potentially related to lupus morbidity and mortality. *G.R. Bruner[1], R.H. Scofield[1,2,3], J.A. Kelly[1], J. Kilpatrick[1], D.K. Klein[1], S.K. Nath[1], T. Lam[1], J.S. Reid[1], J.A. James[1,2], J.B. Harley[1,2,3]*. 1) Arthritis and Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; 2) Department of Medicine, University of Oklahoma, Oklahoma City, OK, USA; 3) US Department of Veterans Affairs Medical Center, Oklahoma City, OK, USA.

In systemic lupus erythematosus (SLE) thrombocytopenia predicts severe disease and an up to 40-fold increased risk of mortality. In 179 multiplex pedigrees with 387 SLE patients, those with thrombocytopenia and SLE are more likely to have nephritis, serositis, neuropsychiatric involvement, autoimmune hemolytic anemia, anti-double stranded DNA, anti-Ro/SS-A and antiphospholipid antibody. In addition, these associated findings are more common in SLE patients without thrombocytopenia within the 38 pedigrees containing an affected with thrombocytopenia than they are in the SLE patients from the remaining 141 pedigrees containing SLE patients with normal platelet counts (OR=5.2, c^2=40.5, $p<0.00001$). Thus, thrombocytopenia identifies families with a severe lupus phenotype. We performed a genome-wide scan on the 38 pedigrees in which at least one SLE-affected member had thrombocytopenia (platelets<100,000 per mcl). Three analytic methods were used: the probability from regressing the mean IBD sharing value against the mean corrected cross-product of the sib-pair trait difference (SIBPAL2), the log probability that affected relatives share alleles IBD (LODPAL), and the lod score from traditional parametric maximum likelihood (ANALYZE). Two linkages were established (of the 308 microsatellite markers evaluated): one at 1q22-24 (lod=3.64) in all 38 pedigrees, and one at 11p13 (lod=4.60) in the 18 African-American pedigrees. In addition, 9 other suggestive genetic effects were found ($p<0.0017$ to 0.0007 or lod>1.9 to 2.2). Thus, these results are consistent with thrombocytopenia being a marker of severe familial SLE, and with genes at 1q22-24 and 11p13 potentially contributing to the high mortality associated with thrombocytopenia in SLE.

Program Nr: 118 from the 2001 ASHG Annual Meeting

Systemic lupus erythematosus and vitiligo: share common gene(s)! *S.K. Nath, J. Kelly, B. Namjou, G. Bruner, R.H. Scofield, C.E. Aston, J.B. Harley.* Arthritis & Immunology, Oklahoma Medical Res Fndn, Oklahoma City, OK.

Systemic lupus erythematosus (SLE), a chronic, complex disease, is the prototype of human systemic autoimmune diseases. During the past few years, there has been considerable interest in identifying genomic segments linked to SLE. Sometimes SLE associates with vitiligo, a common dermatological disorder characterized by hypopigmentary patches that tend to become progressive over time. We identified 18 Caucasian families, multiplex for SLE and each with at least one family member afflicted with both SLE and vitiligo. Genome-wide linkage analyses were conducted using 330 microsatellite markers. Significant evidence for linkage was identified at one chromosomal location (17p), where the maximum multipoint parametric and non-parametric lod scores were 4.27 and 4.31, respectively. The P-values associated with these lod scores was 9.3 x 10-6 and 8.4 x 10-6, respectively. We also performed an affecteds-only analysis by recoding the unaffected individuals as unknown. The affecteds-only lod score of 4.30 was obtained at the same position. We also applied the recently developed conditional logistic regression based non-parametric affected relative pair (ARP) method. The ARP lod score was 4.35 at the same position. In contrast, no evidence of linkage was found in this region when we performed the analysis including all 126 families, unselected for vitiligo, collected for our on going SLE project. We then again recoded new affection status by making individuals affected those who were affected either by SLE or vitiligo. Interestingly, we found an almost similar effect. Therefore, subdividing the SLE families based on the presence of vitiligo may determine a genetically and clinically homogenous group of families in which at least one major gene is segregating that is associated with both clinical phenotypes. Although the genetic and pathophysiological relationship between SLE and vitiligo is unknown, this result suggests that these two autoimmune clinical identities may share a common gene, which is responsible for manifestation of both SLE and vitiligo.

Program Nr: 119 from the 2001 ASHG Annual Meeting

Evidence for a second major histocompatibility complex (MHC) gene involved in the predisposition to familial ankylosing spondylitis (AS). *J. Luo[1], J.M. Akey[1], J. Bruckel[2], J.A. McClain[1], J.D. Reveille[1], L. Jin[1] and For the North American Spondylitis Consortium (NASC).* 1) Environmental Health, University of Cincinnati, Cincinnati, OH; 2) Los Angeles, CA.

Previous studies have suggested that a second MHC gene, located in the class II or III region, may also be involved in susceptibility to Ankylosing Spondylitis (AS). However, the effect of HLA-B27 has confounded the identification of this second gene. To begin to delineate a "second MHC susceptibility gene" for AS, we have genotyped markers dispersed throughout the class I, II, and III regions (8 microsatellite markers and HLA-DRB1, DQA1, and DQB1). For linkage analysis, 104 multiplex AS families containing 146 HLA-B27 positive affected sibling pairs were examined and parametric and nonparametric analyses were conducted with Genehunter. All AS patients met modified New York criteria (1984). HLA-DRB1, DQA1, and DQB1 alleles were ascertained by standard oligotyping procedures with high resolution typing by direct sequencing of exon 2. HLA-B27 typing was carried out in all family members by direct sequence analysis of exons 2 and 3. The maximum NPL score occurred at locus HLA-DRB1 (NPL = 4.93, p = 0.0000007; LOD = 4.93). In conclusion, linkage analyses results strongly suggest the presence of a second MHC gene at or near the HLA-DRB1 locus in predisposition to AS.

Program Nr: 120 from the 2001 ASHG Annual Meeting

Genome-wide scans provide evidence for schizophrenia loci on 1q, 2q and 5q in a nationwide study sample from Finland. *T. Paunio[1], J. Ekelund[1], T. Varilo[1], A. Parker[3], R. Martin[3], J.D. Terwilliger[4], J.S. Sinsheimer[2], T. Partonen[5], J. Lonnqvist[5], J. Meyer[3], L. Peltonen[2]*. 1) Molecular Medicine, NPHI, Helsinki, Finland; 2) Dept of Human Genetics, UCLA, LA, CA; 3) Millennium Pharmaceuticals Inc, Cambridge, MA; 4) Dept of Psychiatry and Columbia Genome Center, Columbia University, NY; 5) Dept of Mental Health and Alcohol Research, NPHI, Helsinki, Finland.

We have previously carried out two genome-wide scans in Finnish study samples ascertained for schizophrenia from national epidemiological registers (Hovatta et al. 1999 and Ekelund et al.2000). Here we report data on fine mapping of 1q 31-42 as a follow-up of these studies as well as data from a third genome scan of the nationwide sample of 238 pedigrees with 589 affected individuals. 53 pedigrees originated from a small internal isolate (IS) on the Eastern border of Finland with well-established genealogical history and small number of founders from 300 years ago. Fine mapping of the 45 cM region on 1q31-42, using 147 polymorphic markers showed strongest evidence for linkage in the vicinity of D1S2709 (Zmax=3.21) in the All-Finland (AF) study sample, and at D1S245, located 38 cM centromeric from D1S2709 in the IS sample (Zmax=2.30). The gamete competition test revealed evidence for linkage and association (P=0.005) in the AF study sample for marker D1S225, 1 cM from D1S2709. Chromosome 1q is emerging as one of the most interesting genome regions for psychosis predisposing loci since many recent genome scans indicate linkage to this region both in schizophrenia and bipolar study samples. The genome scan of over 1,200 individuals using 315 markers revealed two new loci, on chromosome 2q and 5q. For 2q, the best evidence for linkage was obtained in the IS families with D2S427 (Zmax = 4.43) and for 5q with D5S414 (Zmax =3.56) in families originating from the late settlement region. Except for 1q, 2q and 5q, some evidence for linkage emerged from 4q, 9q and Xp, the regions suggested also by our previous scans. The chromosome 5q finding is particularly interesting since several other study samples have shown evidence for linkage in the vicinity of this locus.

Program Nr: 121 from the 2001 ASHG Annual Meeting

Significant evidence for schizophrenia susceptibility loci at 2cen and 10p14 in a large pedigree series. *S.H. Shaw[1], B. Nanthakumar[1], T.J. Crow[2], G. Shields[3], A.B. Smith[3], V.W. Larach[4], N. Wellman[2], J. Loftus[2], K. Razi[3], J. Stewart[3], M. Comazzi[5], A. Vita[5], T. Heffner[6], R. Sherrington[1], L.E. DeLisi[3]*. 1) Axys Pharmaceuticals, La Jolla, CA; 2) University Department of Psychiatry, Warneford Hospital, Oxford, UK; 3) Department of Psychiatry, SUNY, Stony Brook, NY; 4) Department of Psychiatry and Mental Health, University of Chile, Santiago; 5) Department of Psychiatry, University of Milan, Italy; 6) Pfizer, Inc., Ann Arbor, MI.

We have conducted a genome-wide search for schizophrenia susceptibility loci in one of the largest pedigree collections published to date comprising of 293 families each with at least 2 siblings diagnosed with schizophrenia or schizoaffective disorder. Three hundred ninety-six highly polymorphic markers spaced approximately 10cM apart were genotyped in all individuals. Two-point and multipoint non-parametric linkage analyses were performed to evaluate regions of the genome demonstrating increased allele sharing among affected sibling pairs as measured by a lod score. The DSM-III-R defined diagnostic phenotypes for the genetic analysis included 1) schizophrenia-only and 2) schizophrenia plus schizoaffective disorder. Two genomic regions were found with significant multipoint maximum lod scores. On chromosome 2, two peaks occurred flanking the centromere. The first peak in 2p12 occurred between markers D2S139 and D2S417 with a lod score of 4.63 using a schizophrenia-only phenotype. Approximate 17 cM distal in 2q12, a second peak occurred at D2S160 with a lod score of 4.0 using the same diagnostic phenotype. The second significant genomic region occurred on 10p14 with a peak lod score of 3.60 at D10S189 using a schizophrenia plus schizoaffective disorder phenotype. Three other genomic regions were suggestive for linkage: 12q24, 3q27, and 16q12. In addition, marker D22S283 located in 22q12 demonstrated significant increased sharing of maternal alleles in affected individuals (p=0.00005). The entire genome scan with additional parametric analyses in significant genomic regions will be presented.

Program Nr: 122 from the 2001 ASHG Annual Meeting

Gene finding for Parkinson's disease in a genetically isolated population. *P. Heutink[1], M.C.J. Dekker[1], V. Bonifati[1,2], J.J. Houwing-Duistermaat[1], L.A. Sandkuijl[1], J.C. van Swieten[3], B.A. Oostra[1], C.M. van Duijn[1]*. 1) Genetic-Epidemiologic Unit, Departments of Epidemiology & Biostatistics and Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands; 2) Department of Neurological Sciences, La Sapienza University, Rome, Italy; 3) Department of Neurology, Erasmus Medical Center Rotterdam, The Netherlands.

Parkinson's disease (PD) is a common neurodegenerative disorder. The role of genetic factors in PD has long been disputed. However, in recent years several genes involved in Mendelian forms of the disease have been identified. For autosomal recessive early-onset parkinsonism (EOPD) mutations in the *parkin* gene explain up to 50% of cases. Recently a second recessive EOPD locus on chromosome 1p (PARK6) was described. We have identified a genetically isolated population of about 20.000 inhabitants. The population was founded in the middle of the 18th century by a mere 150 settlers and characterized by minimal inward migration and rapid population growth. As part of our research program; Genetic Research in Isolated Populations (GRIP) we ascertained 110 patients with parkinsonism. 57 patients could be related to a common founder 14 generations ago. A systematic genome wide screen was performed using 4 patients with EOPD and 26 patients with idiopathic late-onset PD (LOPD). Homozygosity mapping resulted in significant evidence for linkage (Lod score of 4.3 using MAPMAKER-HOMOZ)to chromosome 1p36 for the EOPD cases. This locus (PARK7) is separated from the PARK6 on chromosome 1p by at least 25 cM. This is the third locus identified so far for EOPD. Using a collection of European families we have determined the relative contribution of the three loci to familial EOPD. Our genome wide screen with LOPD patients showed evidence for allelic association with two adjacent markers on chromosome 10q. Refining of the critical region using additional patients and markers is in progress. The use of genetically isolated populations is increasingly recognized as an important instrument for mapping genes for common and complex disorders and our results clearly demonstrate the feasibility of the GRIP population for finding genes involved in Parkinson's disease.

Program Nr: 123 from the 2001 ASHG Annual Meeting

A Susceptibility Gene for Late-Onset Idiopathic Parkinsons Disease Successfully Mapped. *A. Hicks[1], H. Pétursson[1], T. Jónsson[1], H. Stefánsson[1], H. Jóhannsdóttir[1], J. Sainz[1], M.L. Frigge[1], A. Kong[1], J.R. Gulcher[1], K. Stefánsson[1], S. Sveinbjörnsdóttir[2]*. 1) DeCode Genetics, Reykjavik, Iceland; 2) National university Hospital, Reykjavik, Iceland.

Six regions of the genome (*PARK1-6*) have been implicated in autosomal-dominant and autosomal-recessive forms of early-onset Parkinsons disease. These forms of the disease constitute a minor fraction of all cases. However, until now, no one has successfully mapped a gene contributing to the common late-onset Parkinsons disease. We cross-matched our nationwide genealogy database with a population-based list of Icelandic Parkinsons disease patients to search for families with more than one patient. Using kinship coefficient and relative risk ratio calculations we found strong evidence of a familial component to late-onset disease in Iceland. We have performed a genome-wide scan on 117 patients and 168 of their unaffected relatives within 51 families using 781 microsatellite markers. Allele-sharing, model independent analysis of the results revealed significant linkage to a chromosomal region distinct from all other reported regions, with a lod score of 3.9. By increasing the information content with additional microsatellite markers in this region, the lod score rose to 4.9, which supercedes strict criteria for genome-wide significance. We have thus generated the strongest evidence to date that there is a genetic component in common Parkinsons disease by mapping a gene contributing to the disease. We designate this region *PARK7*. Modelling our data reveals that this locus may contribute to disease susceptibility in a substantial proportion of Icelandic patients. Identification of the gene in this region may pave the way for a better understanding of the disease process, which in turn may lead to improved diagnostics and therapeutics.

Program Nr: 124 from the 2001 ASHG Annual Meeting

A major susceptibility locus for autism spectrum disorders on chromosome 3q25-27 in the Finnish population.

M.P. Auranen[1,2], E. Kempas[1], R. Vanhala[3], T. Varilo[1], T. Ylisaukko-oja[1,2], L. Peltonen[1,2,4], I. Järvelä[1,2,5]. 1) Dept Molecular Medicine, National Public Hlth Inst, Helsinki, Finland; 2) Dept of Medical Genetics, Univ Helsinki, Helsinki, Finland; 3) Unit of Child Neurology, Hospital for Children and Adolescents, Univ Helsinki; 4) Dept of Human Genetics, UCLA, Los Angeles, CA, USA; 5) Lab of Molecular Genetics, Helsinki Univ Hospital, Helsinki, Finland.

Uncovering the genetic basis for autism spectrum disorders has so far been difficult since the diagnoses of the patients are based on behavioural phenotype only with no biochemical measures available. In order to identify predisposing gene loci for autism spectrum disorders we have performed a two-stage genome wide scan in 38 Finnish families with a broad phenotype including infantile autism, Asperger syndrome (AS) and developmental dysphasia. We found a major susceptibility region on chromosome 3q25-27 with a maximum two-point lod score of 4.31 (Zmaxdom) assuming autosomal dominant mode of inheritance in families with infantile autism and AS. Altogether six markers gave Zmaxdom > 3 in the candidate region. The maximum multipoint parametric lod score (MLS) was 4.80 in this region. The significance of the linkage finding was further supported by the identification of a putative ~ 4 cM ancestral haplotype consisting of six markers in 18 families with a broad phenotype. Other potential susceptibility regions identified were 1q21-22, 7q and X with maximum MLS of 2.23, 3.04 and 2.75 respectively. The region on chromosome 1 overlaps with the previously reported candidate region for infantile autism and schizophrenia (Risch et al., 1999; Brzustowicz et al., 2000). On chromosome 7q, Zmaxdom of 3.04 was detected in 19 Finnish families with a broad phenotype.

Program Nr: 125 from the 2001 ASHG Annual Meeting

Identification of Multiple Loci for Alzheimer Disease in an Inbred Israeli-Arab Community. *L.A. Farrer[1], A. Bowirrat[2], R.P. Friedland[3], K. Waraska[1], J.C. Adams[1], A. Korczyn[2], C.T. Baldwin[1].* 1) Boston Univ Sch Medicine, Boston, MA; 2) Tel-Aviv Univ, Israel; 3) Case Western Reserve Univ, Cleveland, OH.

We hypothesized that a high prevalence of Alzheimer disease (AD) in an Israeli-Arab community, which is not associated with the APOE 4 allele, is related to the extensive inbreeding in this community. Historically, Arab populations have proven to be valuable for discovery of new genes. All residents of Wadi Ara, a community of 46,0000 in northern Israel, ages 60 years and older were examined in the home by a neurologist (n=855). AD prevalence rates of 20.5 % among those 60 years or older and 60.5% among those 80 years or older were found. The 4 allele frequency was 3.5% in controls and 4.5 % in AD cases. More than one-third of 168 prevalent cases were from one hamula (extended family) of the 14 found in Wadi Ara, suggesting a genetic basis for AD in this community. A 10 cM genome scan was conducted using DNA's from 5 cases and 5 controls from one hamula. Markers from 8 chromosomal regions showed a significant association with AD ($p < 0.05$). Four of these locations including regions on chromosomes 9 and 12 implicated in genome scans of outbred populations remained significant after follow-up in more than 80 AD cases and 80 controls. Analysis of 12 markers spanning 20 cM on chromosome 9 narrowed the possible location of an AD susceptibility gene to a 10 cM interval between D9S925 and D9S171 (most significant individual result: $p = 0.0005$). Analysis of 11 markers spanning 15 cM on chromosome 12 narrowed the possible location to a 3 cM interval distal to the LRP locus (most significant individual result: $p = 7.3 \times 10^{-12}$). Novel locations on chromosomes 2 ($p = 0.005$) and 8 ($p = 0.000002$) were also identified and are being pursued further. Evidence for linkage on chromosomes 8, 9 and 12 stemmed primarily from excess homozygosity of marker alleles in cases compared to controls suggesting that the genes at these locations behave in either a recessive or additive fashion. The initiative in Wadi Ara will facilitate the identification of novel as well as previously mapped AD genes and provide a unique opportunity to study epistatic and gene-environment interaction.

Program Nr: 126 from the 2001 ASHG Annual Meeting

Genome-wide scan for age at onset of Alzheimer disease. *Y. Li[1], A.M. Saunders[1], A.D. Roses[1], G.W. Small[3], W.K. Scott[1], P.M. Conneally[4], J.M. Vance[1], J.R. Gilbert[1], J.L. Haines[2], M.A. Pericak-Vance[1].* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Vanderbilt University Medical Center Nashville, TN; 3) UCLA Medical Center, Los Angeles, CA; 4) Indiana University Medical Center, Indianapolis, IN.

Alzheimer disease (AD) is the leading cause of dementia in the elderly. Controversy has surrounded whether the effect of genes on late-onset AD is primarily modulating onset or risk. Most studies have modeled the risk of AD as a simple qualitative dichotomous trait. However, examining age-at-onset (AAO) as a quantitative trait can provide novel information and more directly model modulation of onset. We recently performed a genome scan of 455 families for AD risk. We have now analyzed AAO as a truncated quantitative trait using the variance-components approach (SOLAR; Almasy and Blangero, 1998) to identify quantitative trait loci (QTL). 1121/2821 individuals were affected (mean AAO: 72.776.81 years), 742 were unaffected at the time of exam (mean AOE: 70.1813.04 years) and the rest were unknown. Two polygenic models were considered: one included sex as a covariate, the other included sex and apolipoprotein E (APOE) genotypes as covariates. Interestingly, these analyses identified two novel regions and only one region that overlapped with any proposed region harboring risk genes for AD. The three identified regions were found on chromosomes 4 (LOD=2.41 and 2.20 for each model, respectively); 8 (LOD=2.24 and 2.62); and 10 (LOD=2.36 and 2.55). The known effect of APOE on AAO was detected only when the polymorphism itself was tested (LOD=2.92). In general, including APOE as a covariate slightly increased the LOD scores. These data suggest at least one gene (APOE) modulates both risk and onset, while others modulate only onset or risk.

Program Nr: 127 from the 2001 ASHG Annual Meeting

Multiple acyl-coenzyme A dehydrogenase deficiency: acquired leukodystrophy treated with D,L-3-hydroxybutyrate. *J.L.K. Van Hove[1], J. Jaeken[1], L. Lagae[1], P. Demaerel[2], W. Van De Casseye[1], P. Bourdoux[3], K. Niezen-Koning[4]*. 1) Dept Pediatrics, Univ Hosp Gasthuisberg, Leuven, Belgium; 2) Dept Radiology, Univ Hosp Gasthuisberg, Leuven, Belgium; 3) ULB Laboratory of Pediatrics, Free Univ of Brussels, Brussels, Belgium; 4) Research Laboratory, Department of Pediatrics, Groningen, The Netherlands.

This boy, born to consanguineous parents, presented at age 4 months with stridor, swallowing dysfunction, hepatomegaly, myopathy, and cardiomegaly. Brain MRI was normal. Multiple acyl-CoA dehydrogenase deficiency was diagnosed on urine organic acids and blood acylcarnitines. He is homozygous for a point mutation in the ETF dehydrogenase gene. He was successfully treated with frequent low-fat gastrostomy feedings, high-dose L-carnitine, riboflavin, and glycine. He developed almost age-appropriate and could walk independently at age 14 months. However, at age 2 years, he developed a rapidly progressive spastic quadriplegia, leading to complete paralysis in 4 months. Brain MRI showed severe periventricular leukodystrophy. There was no indication for a nutritional deficiency, but the mildly raised CSF lactate rather suggested incomplete metabolic control. Treatment with increasing doses of sodium-D,L-3-hydroxybutyrate, aimed at providing measurable quantities of ketones in serum and CSF, resulted in a decrease in spasticity within a week. Continued treatment over 9 months showed a return of function. He now has good use of arms and hands, can sit independently, and speaks several words again. Brain MRI showed a central clearing of the leukodystrophy with a persistent peripheral leukodystrophic rim, but cystic lesions developed periventricularly. Hepatomegaly, cardiomegaly and transaminases improved. Ketones are an additional therapeutic option for cerebral dysfunction in severe fatty acid oxidation defects.

Program Nr: 128 from the 2001 ASHG Annual Meeting

Oral lysine supplementation reduces plasma ornithine in a mouse model of gyrate atrophy of the choroid and retina (GA). *T. Wang*[1], *G. Steel*[1,2], *D. Valle*[1,2]. 1) Institute of Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 2) Howard Hughes Med Inst.

GA, a blinding, chorioretinal degeneration is an autosomal recessive disorder caused by deficiency of ornithine-d-aminotransferase (OAT) with resulting hyperornithinemia. Previous studies have shown that long term, systemic reduction of plasma ornithine by dietary restriction of arginine prevents retinal degeneration in a GA mouse model (Wang et al PNAS 97:1224,2000) and is beneficial in human GA patients (Kaiser et al Arch Ophthal 109:1539,1991). Unfortunately, <20% of GA patients adhere to the severe dietary restriction required for long-term reduction in plasma ornithine. Overflow ornithinuria in GA results in significant renal loss of other dibasic amino acids (arginine, lysine) due to competition for dibasic transportation in the renal brush border. On this basis, we hypothesized that a high renal filtration of lysine would promote urinary loss of ornithine. We tested this idea on a murine model of GA produced by gene targeting of the *Oat* gene. Adult GA mice (n=6) on a standard amino acid synthetic diet (lysine 1.44%; w/w) were supplemented with lysine up to a total of 6.4%; (w/w, high lys I) or 7.9%; (w/w, high lys II), respectively. Plasma amino acids (mM) were determined weekly. Plasma ornithine levels fell from 1069 (range 894-1275 [nl=76-185]) on the standard diet to 218 (93-351) and 169 (99-216) on high lys I and high lys II, respectively, by week 2 and remained constant thereafter for the 4-6 week study. Plasma lysine levels peaked at 1240 (621-1851) and 1571 (1224-1992) on high lys I and high lys II, respectively, by week 2 (nl=346 [213-572]). There was mild reduction (20-35%) in plasma arginine. The animals had a mild weight loss (~10%) during week 1 and regained baseline weight by week 2. Long term studies on the metabolic and retinal phenotype of these animals on diets are in progress. Our studies indicate that oral lysine supplementation effectively reduces plasma ornithine in GA mice and suggest that a combination of dietary lysine supplementation and arginine restriction may improve the current dietary management in GA patients.

Program Nr: 129 from the 2001 ASHG Annual Meeting

Metabolic Profiling in Succinate Semialdehyde Dehydrogenase (SSADH) Deficient Mice Provides Insight into Neuro(patho)mechanims. *K.M. Gibson[1], D.S.M. Schor[2], W. Guerand[2], T. Bottiglieri[3], T. Burlingame[1], B.M. Hogema[1,2,4], M. Gupta[1], H. Bartels[1,5], W. Froestl[6], O.C. Snead[7], M. Grompe[1], C. Jakobs[2]*. 1) Biochem Gen Lab, Molec/Med Gen, Oregon Health Sci Univ, Portland, OR; 2) Clin Chem, Free Univ, The Netherlands; 3) Inst of Metab Dis, Baylor Univ Med Ctr, Dallas, TX; 4) Erasmus Univ, Rotterdam, The Netherlands; 5) Univ Groningen School of Medicine, Groningen, The Netherlands; 6) Novartis Pharma, Basle, Switzerland; 7) Dept Neurol, Hospital for Sick Children, Toronto, Ontario, Canada.

SSADH is the second enzyme of GABA catabolism following GABA-transaminase (GABA-T). To elucidate pathomechanisms in human SSADH deficiency, we developed mice with targeted deletion of the SSADH gene (-/- mice). These animals (like their human counterparts) were expected to accumulate gamma-hydroxybutyric acid (GHB), a compound with unusual neuropharmacologic properties and an expanding drug of abuse. We determined GHB, total GABA (tGABA) and tissue amino acid profiles (brain, liver, kidney, heart, pancreas) in -/- mice. Beta-alanine (bAla), a GABA homologue and putative neurotransmitter proposed to be metabolized by GABA-T, was also determined. For control, we found (umol/g protein; range of tissue means): GHB, 0.05-0.24; tGABA, 0.11-1.51 (brain 49.9); and bAla, 1.05-4.28. Except for bAla, all concentrations were increased in all tissues of -/- mice (GHB, 19-46 fold; tGABA, 3-22 fold); for bAla, only kidney and liver showed significant elevations. Amino acid analysis in liver, kidney and brain revealed elevated GABA in all tissues. Brain glutamine levels were significantly decreased in -/- mice (control 41 +/- 7; -/- mice 11 +/- 8 umol/g protein (mean +/- SD); all other physiologic amino acid levels in all tissues were comparable to control. We conclude that 1) bAla is unlikely to play a significant role in neuropathology associated with SSADH deficiency; 2) liver and kidney GABA-T catabolizes both GABA and bAla, whereas different transaminases may catalyze these reactions in other tissues; and 3) decreased brain glutamine levels suggest loss of glutamine synthase activity and astrocyte damage in the brain of -/- mice. (supported by NS 40270 and March of Dimes #1-FY00-352).

Program Nr: 130 from the 2001 ASHG Annual Meeting

The deficiency of the ocrl1 PIP2 5-phosphatase in Lowe syndrome affects actin polymerization. *S.F. Suchy, R.L. Nussbaum.* Genetic Diseases Res Branch, NHGRI/NIH, Bethesda, MD.

The oculocerebrorenal syndrome of Lowe is a rare X-linked disorder characterized by bilateral congenital cataracts, renal Fanconi syndrome and mental retardation. Lowe syndrome is due to a deficiency of the ocrl1 protein, a phosphatidylinositol 4,5- bisphosphate (PIP2) 5-phosphatase localized to the *trans*-Golgi network. At present, it is not known how a defective PIP2 5-phosphatase produces the lens, kidney and brain phenotype in Lowe syndrome. The substrate for this phosphatase, PIP2, is an important lipid modulator of cellular processes such cell signaling, protein trafficking and actin polymerization. PIP2 has been consistently demonstrated to be a positive effector of actin polymerization *in vitro*. We report here the novel finding that Lowe fibroblasts have a reduction in actin stress fibers and respond more rapidly to depolymerizing agents than control cells. These results appear to differ from in vitro data in which elevated PIP2 levels increase actin polymerization. In addition to the reduced actin stress fiber staining, we also show that Lowe cells have an increase in punctate F-actin staining in the center of the cell, that co-localizes with the actin severing and capping protein, gelsolin. This punctate F-actin/gelsolin staining, absent in normal fibroblasts, is concentrated in a central region of the cell and does not coincide with the localization of *trans*-Golgi network markers. Interestingly, the actin severing activity of gelsolin is regulated by both PIP2 and Ca^{2+} that would be expected to be altered in Lowe cells. The discovery of a cellular actin phenotype in Lowe syndrome appears relevant to the clinical phenotype since actin polymerization plays a key role in the formation, maintenance and proper function of tight junctions and adherens junctions. These cell-cell contacts have been demonstrated to be critical in renal proximal tubule function, and in the differentiation of the lens. Furthermore, it has been shown that these contacts fail to form when actin polymerization is disrupted. These findings point to a general mechanism to explain how a PIP2 phosphatase deficiency might produce the Lowe syndrome phenotype.

Program Nr: 131 from the 2001 ASHG Annual Meeting

Farnesylation of Pex19p is essential for peroxisome biogenesis in *Saccharomyces cerevisiae.* . *S. Almashanu, D. Valle.* Howard Hughes Medical Institut, Institute of Genetic Medicine, Johns Hopkins University.

Pex19p, a mainly cytosolic, farnesylated protein, is essential for peroxisome biogenesis, possibly serving as a receptor for peroxisome membrane proteins (PMP). Currently, the role of the farnesyl group in Pex19p function is unknown. Expression of nonfarnesylated Pex19p, in some experimental settings, only partially complemented pex19D cells and farnesylation seems to be required for Pex19p interaction with PMP. By contrast, in other experimental settings the farnesyl moiety seems dispensable for Pex19p function. Pex19p, like other prenylated proteins, has a C-terminal CAAX motif that, following farnesylation of the C residue, presumably undergoes cleavage of the AAX and carboxyl-methylation of the C residue. This pathway in *S. cerevisiae* involves five genes encoding a cytosolic heterodimeric farnesyl-protein transferase (Ram1p/Ram2p), and three ER membrane proteins (two endoproteases, Rce1p and Ste24p, and one carboxyl-methyltransferase, Ste14p). To determine the requirement for farnesylation of Pex19p in peroxisome biogenesis we examined peroxisome biogenesis in *S. cerevisiae* strains, each deleted for one or more components of the farnesylation pathway but all wild type for *PEX19*. We tested these strains for the localization of a peroxisomal matrix protein marker (GFP/PTS1) and a marker for PMP (PEX13p/GFP). We also determined the distribution of the peroxisomal matrix protein catalase. We find that ram1D cells have defective peroxisome biogenesis identical to that of a pex19D strain, including absence of remnant peroxisomes and the mislocalization of catalase to the cytosol. However, the ste14D strain, deleted for the carboxyl-methyltransferase, as well as, strain deleted for the proteases (rce1Dste24D) have normal peroxisomes. These results indicate that the first step of the farnesylation pathway is essential for peroxisome biogenesis and strongly support the contention that farnesylation is essential for Pex19p function. We hypothesize that mutation in a mammalian farnesyl-protein transferase may include a peroxisome biogenesis defect as part of its phenotype.

Program Nr: 132 from the 2001 ASHG Annual Meeting

Maleylacetoacetato isomerase knock out mouse reveals a non-enzymatic GSH-mediated bypass for MAAI enzymatic activity. *J.M. Fernández-Cañón[1], T. Burlingame[1], M. Finegold[2], M. Gibbson[1], M. Grompe[1]*. 1) Dept Mol. & Med. Gen., Oregon Health Sci Univ, Portland, OR; 2) Texas children's Hospital. Houston. TX.

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) and accumulation of fumarylacetoacetate (FAA) which is converted to succinylacetoacetate (SAA) and succinylacetone (SA). This is the diagnostic compound for HT1. We have generated a mouse knocked out for MAAI activity. Deficient mice accumulate FAA and SA in the urine but they are healthy and breed normally. It may be the MAA (or any accumulated derivative) is not toxic or there is a bypass for MAAI activity. However, the mutants were sensitive to overload the phenylalanine/tyrosine catabolic pathway. After IP injection of >15 mg of homogentisic acid MAAI mutant mice died in 3-12 hours. Addition of phenylalanine or tyrosine to the diet also resulted in rapid death. Liver function test indicated acute liver failure (elevation of ALT and bilirubin) and histopathology confirmed liver and kidney damage. The expression of genes induced by oxidative stress (NMO-1) was increased, but different to FAH mutant mice the expression of CHOP-1 (marker for DNA damage) is almost normal. These facts are indicating that there is toxicity resulting from MAAI deficiency. We also found a bypass for MAAI activity. Double mutant MAAI/FAH died in 5-10 days when OFF NTBC. These mutants have a high increase of creatine in blood which is indicating kidney damage. Kidneys should be the primary target for the accumulated compound/s resulting from MAAI deficiency. We can assay a GSH-mediated isomerization of MAA to FAA which is independent of MAAI enzyme but dependent of GSH (other thiol molecules, as DTT, are less efficient to promote this isomerization). This bypass should be responsible for the lack of toxicity in MAAI mutant mice in regular diet.

Program Nr: 133 from the 2001 ASHG Annual Meeting

Late-onset cortico-hippocampal neurodepletion due to catastrophic failure of oxidative phosphorylation in MILON mice. *N.G. Larsson[1], L. Sorensen[1], M. Ekstrand[1], J. Silva[1], E. Lindqvist[2], B. Xu[3], P. Rustin[4], L. Olson[2]*. 1) Medical Nutrtion, Karolinska Institutet, Huddinge, Sweden; 2) Department of Neuroscience, Karolinska Institutet, S-171 77 Stockholm, Sweden; 3) Department of Physiology, Howard Hughes Medical Institute, University of California, San Francisco, California 94143, USA; 4) Unite de Recherches sur les Handicaps Genetiques de l'Enfant, INSERM U393, Hpital des Enfants-Malades, Paris, France.

We have generated mitochondrial late onset neurodegeneration (MILON) mice with postnatal disruption of oxidative phosphorylation in forebrain neurons. They develop normally and display no overt behavioral disturbances or histological changes during the first 5 months of life. The MILON mice display reduced levels of mitochondrial DNA (mtDNA) and mitochondrial RNA (mtRNA) from 2 and 4 months of age, respectively, and severely respiratory-chain-deficient neurons from 4 months of age. Surprisingly, these respiratory-chain-deficient neurons are viable for at least one month without showing signs of neurodegeneration or major induction of defenses against oxidative stress. Prolonged neuronal respiratory chain deficiency is thus required for the induction of neurodegeneration. At about 5-5.5 months of age MILON mice start to show signs of disease followed by death shortly thereafter. The debut of overt disease in MILON mice coincides with onset of rapidly progressive neurodegeneration and massive cell death in hippocampus and neocortex. This profound neurodegenerative process is manifested as axonal degeneration, gliosis and abundant TUNEL-positive nuclei. The MILON mouse model provides a novel and powerful tool for further studies of the role for respiratory chain deficiency in neurodegeneration and aging.Late-onset cortico-hippocampal neurodepletion due to catastrophic failure of oxidative phosphorylation in MILON mice Late-onset cortico-hippocampal neurodepletion due to catastrophic failure of oxidative phosphorylation in MILON mice Late-onset cortico-hippocampal neurodepletion due to catastrophic failure of oxidative phosphorylation in MILON mice.

Program Nr: 134 from the 2001 ASHG Annual Meeting

Reduced very long-chain acyl-coA synthetase activity decreases VLCFA b**-oxidation but does not alter the ALD mouse phenotype.** *A.K. Heinzer[2], J.F. Lu[1], P.A. Watkins[1], K.D. Smith[1,2]*. 1) Kennedy Krieger Institute, Baltimore, MD; 2) Institute for Genomic Medicine, Johns Hopkins University, Baltimore, MD.

X-linked adrenoleukodystrophy (XALD) is characterized by an accumulation of saturated very long-chain fatty acids (VLCFA) (C22:0 and greater) that has been attributed to reduced peroxisomal VLCFA b-oxidation and reduced peroxisomal very long chain acyl-coA synthetase (VLCS) activity which catalyzes the first step in VLCFA b -oxidation. The ALD mouse accumulates VLCFA in tissues but tissue VLCFA b -oxidation is not decreased. Here we describe a VLCS knockout mouse and the effect of this mutation on the ALD mouse. VLCS is highly expressed in liver and kidney, and in VLCS mice the ability to activate VLCFA in these tissues is reduced by 80%, and the ability to b -oxidize VLCFA is decreased by 50% as compared to wild type. However, this reduction in degradation of VLCFA does not result in an accumulation of VLCFA. This uncoupling of VLCFA accumulation and degradation in both VLCS and ALD mice is unsettling in the light of current explanations of XALD pathophysiology. VLCS knockout mice are viable, fertile, display no gross physical abnormalities, and are histologically no different from wild type controls. A VLCS/ALD double knockout mouse is also viable, fertile, and shows no sign of neurological deficits at 12 weeks of age. This strain's ability to b -oxidize VLCFA, VLCFA accumulation, neurological disability, life span, and response to various diets high in saturated VLCFA, branched chain fatty acids, and total fat content will be discussed.

Program Nr: 135 from the 2001 ASHG Annual Meeting

Investigation of the role of the mannose-6-phosphate receptor (MPR300) in lysosomal enzyme uptake *in vivo* using tissue-specific MPR300 knockout mice. *A.J. McVie-Wylie[1], A.A. Wylie[2], D. Lamson[1], J. Dai[1], R.L. Jirtle[2], Y.T. Chen[1]*. 1) Division of Medical Genetics; 2) Division of Radiation Oncology, Duke University Medical Center, Durham, NC, 27710.

The lysosomal storage diseases (LSD) are amenable to enzyme replacement (ERT) and gene therapy through receptor-mediated endocytosis. Fabry disease, Mucopolysaccharidoses and Pompe disease are examples of LSD in which ERT was developed to target the affected organs via the mannose-6-phosphate receptor (MPR300). *In vitro* studies have established that the MPR300 is necessary for lysosomal enzyme uptake, however it is presently unclear if the same system is involved *in vivo*. Complete ablation of MPR300 expression in transgenic mice is lethal; therefore, we have generated viable tissue-specific MPR300 knockout mice to investigate this question and to better define the role of the receptor *in vivo*. Two strains of tissue-specific MPR300 knockout mice (KO) were generated: one in the liver and the other in the muscle. Both strains are viable, fertile and without obvious phenotypes, including absent organomegaly. Western blot analysis indicates that the mice demonstrate markedly decreased MPR300 protein in the tissues affected (>90% compared to wild type), while maintaining normal levels elsewhere. Injections of the lysosomal enzyme acid a-glucosidase (GAA, deficient in Pompe disease) into the liver specific KO mouse resulted in a 30% decrease of enzyme uptake in the liver when compared to age-matched controls. This indicates that the MPR300 does indeed play a significant role in enzyme uptake in this tissue *in vivo*. Similar injections were undertaken with the muscle specific KO mice, however background levels of murine GAA in this tissue interfered with our interpretations of the results. To eliminate this problem we are generating muscle specific MPR300 KO and GAA deficient double transgenic mice. In conclusion, the tissue-specific MPR300 KO mice will be effective tools to better define the mechanism of lysosomal enzyme uptake *in vivo*, and this will ultimately provide scientists with a means to improve the efficacy of the therapy currently available for LSD.

Program Nr: 136 from the 2001 ASHG Annual Meeting

Defective non-shivering thermogenesis triggered by inappropriate activation of PPARa in the 70kDa Peroxisomal Membrane Protein (PMP70) deficient mice. *I. Silva-Zolezzi[1,2], K.J. Hebron[1], S.J. Mihalik[3], D. Valle[1], G. Jimenez-Sanchez[1]*. 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Programa Doctoral en Biomedicina Molecular, CINVESTAV, Mexico; 3) Kennedy Krieger Institute, Baltimore, MD.

ATP-binding cassette (ABC) transporters comprise a superfamily of membrane proteins involved in the transport of a variety of molecules across biological membranes. There are four known human peroxisome half ABC transporters: PMP70, ALDP, ALDR and P70R. Mutations in *ALD* cause X-linked Adrenoleukodystrophy. The function of the other three is unknown. We produced PMP70 knockout mice (PMP70-/-) by standard methods. These mice are viable and have peroxisomes with normal PTS1 and PTS2 import systems; dramatic (10X) reduction in hepatic glycogen in the fed state; striking medium chain dicarboxylic aciduria (C8, C8:1, C9, C10, C10:1) that increases during the fasted state; defective phytanic and pristanic acids oxidation (~50%), resulting in a 10X plasma accumulation of these molecules after a phytol supplemented diet; and defective non-shivering thermogenesis (DNST), with a drop in body temperature to <15^{o}C after 2-6hr at 4^{o}C (Controls 34±2^{o}C), similar to mice with defects in mitochondrial b-oxidation and rats fasted for >24hr. Our results suggest that deficiency of PMP70 results in accumulation of phytanic and pristanic acids with a secondary activation of PPARa, leading to inappropriate activation of fasting fuel homeostasis. In support of our hypothesis we found increased expression of the PPARa target genes *MCAD, ACO, ACAA1, FABP* and *CYP4A3*. White adipose tissue was reduced by 30%; in the PMP70-/- mice compared to controls while brown adipose tissue (BAT) showed no significant size difference. Light microscopy of BAT showed that tissue architecture and fat content were similar in the PMP70-/- animals and controls after 0, 1.5, 3 and 6hr at 4^{o}C while mice with inherited defects in mitochondrial b-oxidation show accumulation of fat. These results indicate that DNST in the PMP70-/- mice occurs despite normal mitochondrial b-oxidation and suggest that inappropriate activation of PPARa impairs heat production in BAT.

Program Nr: 137 from the 2001 ASHG Annual Meeting

rent1, a mammalian *trans*-effector of nonsense-mediated mRNA decay, shuttles between the nucleus and cytoplasm. *J.T. Mendell[1], H.A. Laken[1], H.C. Dietz[1, 2].* 1) Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Howard Hughes Medical Institute.

Transcripts harboring premature termination codons (PTCs) are rapidly degraded in eukaryotic cells through a process known as nonsense-mediated mRNA decay (NMD). Although this pathway has the potential to influence mutant transcript expression for nearly 1/3 of human disease-causing alleles, relatively little is known about its basic mechanism. For example, the cellular location of nonsense surveillance remains obscure, given that both cytoplasmic and nuclear functions (translation and splicing, respectively) are required. Prior examination of the subcellular localization of human proteins required for NMD (rent1, rent2, and hUpf3) has documented that rent1 and rent2 localize to the cytoplasm, while hUpf3 is predominantly nuclear and shuttles between cellular compartments. We have further investigated the subcellular localization of rent1 utilizing leptomycin B (LMB), a specific inhibitor of CRM1-mediated nuclear export. Upon treatment with the drug, we observed dramatic nuclear accumulation of both endogenous rent1 and a recombinantly expressed rent1-GFP fusion protein. The subcellular localization of a series of rent1-GFP fusion proteins containing overlapping deletions was determined in the presence and absence of LMB, allowing identification of domains required for nuclear import and export. Neither of these newly defined domains contain sequences matching previously described nuclear import or export signals. We demonstrate, however, that RanBP7, an importin-b homologue and nuclear import receptor for selected ribosomal proteins, interacts with rent1. Furthermore, deletion of the rent1 nuclear import domain abolishes RanBP7 interaction. These data document that rent1 shuttles between the nucleus and cytoplasm and implicate RanBP7 as the rent1 nuclear importer. The presence of rent1 in the nucleus presents an opportunity for the NMD machinery to participate in nuclear nonsense surveillance. This would provide a mechanism for previously described PTC-mediated perturbations of nuclear nonsense transcripts including abnormalities in stability, splicing, and trafficking.

Program Nr: 138 from the 2001 ASHG Annual Meeting

Perturbation of thymic development in nonsense-mediated decay-deficient mice. *P.A. Frischmeyer[1], R.A. Montgomery[2], S.K. Cooke[2], D.S. Warren[2], C.J. Sonnenday[2], H.C. Dietz[1].* 1) HHMI/Inst. of Genetic Med., Johns Hopkins SOM, Balt., MD; 2) Dept of Surgery Johns Hopkins SOM, Balt., MD.

The ability to detect and degrade transcripts that harbor premature termination codons (PTCs) is universal among eukaryotes. This surveillance mechanism, termed nonsense-mediated mRNA decay (NMD), is a potent modulator of many disease phenotypes caused by nonsense mutations. Recent data suggest that the pathway also regulates the stability of selected physiologic transcripts. Mammalian NMD requires the activities of three *trans*-acting factors termed *RENT1-3*. Although absence of NMD in yeast and worms is well tolerated, targeted disruption of *RENT1* in mice resulted in complete loss of NMD and proved incompatible with embryonic viability. Transgenic (Tg+) mice that ubiquitously express a dominant negative form of rent1 that causes a relative (approx. 3-fold) stabilization of nonsense transcripts have a normal life span and no gross phenotypic abnormalities but demonstrate impaired T-cell maturation. T-cell receptor repertoire diversity is generated by the imprecise joining of TCR gene segments which results in 2 out of 3 allelic rearrangements containing a PTC. During normal fetal thymic ontogeny, thymocyte expression of the CD4/CD8 co-receptors correlates temporally with TCR-b allele rearrangement and expression. FACs analysis of thymocytes at 16 and 17 dpc revealed a dramatic paucity of double positive cells in Tg+ animals relative to WT littermates. Sequence analysis documented a diminished frequency of thymocytes harboring nonproductive TCR-b rearrangements. Histologic analysis of thymi from NMD-deficient mice demonstrated discrete zones of cellular drop-out that showed intense apoptosis by TUNEL staining, culminating in diffuse glandular atrophy by the time of birth. Peripheral consequences include a reduced number of circulating lymphocytes and expansion and coalescence of splenic follicles. Our model system provides the first opportunity to explore the cellular and phenotypic consequences of loss of NMD efficiency in higher eukaryotes. These data will prove relevant during the development of therapeutic strategies based upon manipulation of NMD and nonsense suppression.

Program Nr: 139 from the 2001 ASHG Annual Meeting

Detectable consequences of synonymous SNPs in the human dopamine D2 receptor gene. *P.V. Gejman[1], J. Duan[1], M. Antezana[1], J. Comeron[1, 2], A. Sanders[1]*. 1) Dept Psychiatry, Univ Chicago, JF Knapp Res Ctr, Chicago, IL; 2) Department of Ecology and Evolution, , Univ Chicago, Chicago, IL.

Six synonymous SNPs are known from the coding region of the dopamine receptor D2 (DRD2) gene: C132T, G423A, T765C, C939T, C957T, and G1101A. Preliminary analyses showed that DRD2 codon preferences depart from isochoric nucleotide frequencies and that the aforementioned mutations affect the folding of the DRD2 mRNA predicted by the program, MFOLD. This indicated that some of the synonymous changes might affect the function of the gene. We therefore determined empirically whether these synonymous changes affect mRNA folding, half-life and translatability. We constructed expression vectors carrying each synonymous mutation separately, and a vector carrying two of them (957T and 1101A) in an otherwise wild-type DRD2 background sequence. The folding pattern of mRNAs transcribed in vitro from these vectors was probed with nuclease S1 confirming some aspects of the computer-predicted foldings. Translation experiments in a cell-free system showed that the mRNA carrying 957T had decreased translatability relative to the wild-type. This mRNA had also a shorter half-life and was less responsive to up-regulation by extracellular dopamine. The results prove that synonymous mutations in DRD2 can have empirically measurable biological consequences that might be of physiological and pharmacological significance. To optimize the search for further such mutations we are in the process of testing the hypothesis that base changes that produce the maximum change in computer-determined mRNA folding energy are also those with the most extreme functional consequences. To this end we calculated the folding energies of mRNAs carrying, one at the time, each of the three possible point mutations at each site of the DRD2 coding sequence. We will synthesize and characterize functionally those with the most extreme deviations in energy from the wild-type. We will also study the functional effects of modifying the major codon content and the off-frame composition of the DRD2 coding region by synthesizing de novo sequences with appropriate base changes and characterizing them in vitro.

Program Nr: 140 from the 2001 ASHG Annual Meeting

Altering the downstream 5' splice site corrects missplicing of an exon caused by an abbreviated polypyrimidine tract. *T.W. Hefferon[1], F.C. Broackes-Carter[2], A. Harris[2], G.R. Cutting[1]*. 1) Inst. of Genetic Medicine, Johns Hopkins Univ., Baltimore, MD; 2) Weatherall Inst., Univ. of Oxford, England.

A major question to be answered in molecular biology is why some exons are misspliced and not others. A well-studied example of missplicing is exon 9 of the human cystic fibrosis transmembrane conductance regulator gene (CFTR), in which an abbreviated upstream polypyrimidine (Py) tract is associated with aberrant splicing and disease. However, many introns have abbreviated Py tracts, yet do not missplice. Inspection of the 5' splice sites up- and downstream of exon 9 revealed deviations from consensus sequence, so we hypothesized that this exon may be inherently vulnerable to missplicing. To test this idea, we constructed a CFTR minigene and replicated missplicing caused by a shortened 5 thymidine Py tract upstream of the exon (69.4±9.1% exon 9+ transcripts, n=8). Replacement of a nonconsensus pyrimidine with a consensus purine in the intron 8 5' splice site resulted in increased missplicing (53.4±3.4, n=10) due to the modified intron 8 5' splice site outcompeting the native intron 9 5' splice site. Indeed, insertion of a consensus adenine at position +3 in the intron 9 5' splice site resulted in near normal splicing (98.3±2.3, n=10) regardless of whether the intron 8 5' splice site was modified or native. Our hypothesis was supported by data from the sheep and mouse. Although sheep CFTR exon 9 is preceded by a long Py tract (Py_{14}), it has a nonconsensus intron 9 5' splice site which is identical to human, and we demonstrated that the sheep missplices *in vivo*. On the other hand, mouse CFTR exon 9 is preceded by a short Py tract (Py_5) but is not misspliced. Its intron 9 5' splice site differs from human by a two-nucleotide insertion which, when introduced into our minigene, repaired splicing (98.6±2.1, n=4). Together, these observations place new emphasis on deviations at 5' splice sites in nucleotides other than the invariant GT, particularly when found in conjunction with altered splicing sequences such as a shortened Py tract. Thus careful inspection of nucleotides at the +3 to +6 position of downstream 5' splice sites may identify exons which are vulnerable to missplicing.

Program Nr: 141 from the 2001 ASHG Annual Meeting

Identification of trans-factors that promote inclusion of the alternatively spliced exon 7 of SMN2 as a basis for therapy in patients with spinal muscular atrophy. *B. Wirth, Y. Hofmann.* Dept Molecular Genetics, Inst Human Genetics, Bonn, Germany.

Spinal muscular atrophy (SMA), the second most frequent autosomal recessive disorder in human is a motor neuron disease that results from loss of functional survival motor neuron (SMN1) alleles. Ninety-six percent of SMA patients display homozygous deletions/mutations in SMN1. Homozygous absence of the nearly identical copy gene SMN2 has no clinical phenotype but the copy number influences the severity of SMA. However, SMN2 fails to provide protection from SMA due to a single translationally silent nucleotide difference in exon 7. This disrupts an exonic splicing enhancer (ESE) and causes exon 7 skipping leading to abundant production of a shorter isoform, SMN2delta7. The truncated transcript encodes a less stable protein with reduced self-oligomerization activity that fails to compensate for the loss of SMN1. Last year, we identified the first in vivo regulator of SMN mRNA processing, Htra2-beta1, which is a splicing factor that promotes the inclusion of SMN exon 7 and stimulates full-length SMN2 expression to almost 90%. Htra2-beta1 specifically functions through and binds an AG-rich ESE in SMN exon 7. Through functional splice assays we identified two further, although weaker, trans-factors that stimulate the inclusion of SMN2 exon 7 in a dose-dependant manner, resulting in ~40% and 54% of full-length transcripts, respectively. Moreover, these factors interact with Htra2-beta1 as shown by in vivo co-immunoprecipitation and support spliceosome assembly at the weak 3' splice site as demonstrated by functional co-splice assays. Interestingly, a constant low amount of Htra2-beta1 and increasing amounts of these new trans-factors resulted in approximately the same rate of SMN2 full-length transcript as the highest amount of Htra2-beta1 tested. The identification of the whole complex that modulates the splicing pattern of SMN2 might be helpful for the development of a somatic gene therapy in SMA patients, since each SMA patient retains at least one SMN2 copy that might be used to produce sufficient amounts of full-length SMN2.

Program Nr: 142 from the 2001 ASHG Annual Meeting

Lmx1b is required for podocyte differentiation and foot process morphogenesis. *R. Morello[1], J.H. Miner[2], B. Lee[1].* 1) Dept Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept Medicine, Washington University School of Medicine, St. Louis, Mo.

LMX1B is a Lim-homeodoamin transcription factor. We have demonstrated that mutations in *LMX1B* cause Nail Patella Syndrome (NPS), an autosomal dominant disease characterized by skeletal abnormalities, hypoplastic nails, and nephropathy. Recently we have shown an alteration in the expression of Type IV Collagen in the glomerular basement membrane (GBM) of *Lmx1b-/-* mice, a mouse model for NPS. Lmx1b is able to bind to an enhancer-like sequence in the first intron of *Col4a4* regulating both *Col4a4* and *Col4a3* expression during metanephric kidney development. These extracellular molecules represent the first identified direct transcriptional targets of *Lmx1b*. This architectural alteration of the GBM, is also observed in NPS patients and contributes to defective glomerular filtration characterized by early onset proteinuria and hematuria. Based on alteration of podocyte morphology in EM studies of NPS kidney biopsies we hypothesized that loss of *Lmx1b* might cause a more generalized defect of podocytes differentiation. Our EMs of control and mutant glomeruli show that *Lmx1b* mutant podocytes have dysplastic foot processes. The few cell-cell junctions that are visible do not have typical slit diaphragms and the podocytes have arrested development. We examined the expression of markers of podocyte differentiation in wild-type and *Lmx1b-/-* newborn mouse kidneys, including Nephrin, Cd2ap, Synaptopodin, Podocin, Zo1, integrin, and laminin. Our data show strongly decreased expression of both Cd2ap and Podocin on RNA and protein levels, suggesting an important role of these molecules for the proper organization of the slit diaphragm. Furthermore, we identified several Lmx1b binding sites in both Cd2ap and Podocin promoters and demonstrated that Lmx1b is able to specifically bind to these sequences. Hence, *Lmx1b* is a key transcription factor regulating multiple markers of podocytes maturation. Loss of *Lmx1b* results in disruption of foot process maturation and GBM formation and explains the more severe nephropathy observed in NPS patients compared to Alport syndrome patients.

Program Nr: 143 from the 2001 ASHG Annual Meeting

The Leucine Zipper of the ATM protein regulates cellular sensitivity to DNA damage. *S. Chen, B.D Price.* Dept Adult Oncology, D810, Dana-Farber Cancer Inst, Boston, MA.

The human disease Ataxia Telangiectasia (AT) is characterized neurodegeneration, increased incidence of cancer, immunodeficiency and hypersensitivity to Ionizing Radiation (IR). ATM, the product of the AT gene, is a protein kinase which phosphorylates several DNA repair proteins, including p53, Brca1 and nibrin. ATM contains a putative Leucine Zipper (LZ: residues 1216-1239). LZs regulate homo- and hetero-dimerization of proteins, and are found in proteins, which regulate gene transcription. We have deleted the LZ from the ATM protein, generating ATDLZ. AT cells stably expressing vector, full-length ATM (FLAT) or ATDLZ were constructed. Expression of FLAT in ATM negative cells caused increased cell survival following exposure to IR, whereas expression of ATDLZ failed to increase cell survival. Thus the LZ of ATM is required for ATM to regulate cell survival following exposure to IR. In addition, the LZ of ATM does not regulate the formation of ATM homo-dimers, suggesting that the LZ regulates interaction with other cellular signaling proteins. Amino-acids 773-1364 of ATM, containing the LZ, were expressed as a dominant negative fragment in SW480 cells, which express normal levels of ATM protein. Amino-acids 773-1364 sensitized SW480 cells to IR. However, when the LZ was deleted from this fragment, it was still able to sensitize SW480 cells to IR. Thus ATM contains multiple domains which regulate cell survival following exposure to IR. Further, our data provide strong evidence to indicate that the LZ is essential for ATM in regulating response to IR.

Program Nr: 144 from the 2001 ASHG Annual Meeting

Dp260 isoform of dystrophin is able to bind actin and produces a Becker-like phenotype in transgenic *mdx* mice.

L.E. Warner[1], C. Dello Russo[1], R.W. Crawford[1], J.M. Ervasti[2], J.S. Chamberlain[1]. 1) Dept Neurology, Univ Washington, Seattle, WA; 2) Dept Physiology, Univ Wisconsin, Madison, WI.

Duchenne muscular dystrophy (DMD) is caused by defects in the dystrophin gene. DMD is clinically characterized by severe, progressive muscle degeneration and weakness. In muscle, dystrophin is thought to play a mechanical role linking the actin cytoskeleton to the extracellular matrix through its assembly in a larger complex called the dystrophin glycoprotein complex (DGC). This link helps maintain muscle membrane integrity in part by dissipating the forces of muscle contraction into the extracellular matrix. Loss of the DGC results in contraction-induced injury and muscle degeneration in *mdx* mice and DMD patients. Dystrophin has an N-terminal actin-binding domain (ABD) as well as an internal ABD within the central rod region (repeats 11-17). Deletion of the internal ABD does not significantly affect the ability of dystrophin to bind actin or prevent dystrophy. However, it is not known whether the internal ABD alone could bind actin *in vivo* and reverse the dystrophic phenotype in *mdx* mice. To test this concept, we constructed transgenic *mdx* mice expressing Dp260 in skeletal muscle. Dp260 is the retinal-specific isoform of dystrophin and lacks the N-terminal ABD and the first 9 repeats of the rod region, but retains the internal ABD. Our results indicated that the internal ABD is able to bind actin in the absence of the N-terminal ABD and with similar affinity. While Dp260 did not fully prevent dystrophy in *mdx* mice, it slowed progression of the disease and prevented development of inflammation and fibrosis. The difference in functionality between constructs deleted for the two ABD may be related to differences in specificity of actin binding and the particular repeats deleted. Importantly, Dp260 was highly effective in protecting muscles from contraction-induced injury, demonstrating a mechanically functional link with the cytoskeleton. In contrast, force development in transgenic muscle was not different from *mdx* muscles. These results suggest that the dystrophin rod domain may facilitate the formation of a muscle architecture optimized for maximal force development.

Program Nr: 145 from the 2001 ASHG Annual Meeting

A novel gene for Bartter syndrome with sensorineural deafness and kidney failure is expressed in adult and embryonic kidney. *R. Birkenhager[1], E. Otto[1], M.J. Schuermann[1], E.-M. Ruf[1], A. Kispert[2], F. Hildebrandt[1].* 1) Univ Children`s Hosp., Univ Freiburg, Freiburg, Germany; 2) Instiute for Molecular Biology, Medizinische Hochschule Hannover, Germany.

Antenatal Bartter syndrome (aBS) is an autosomal recessive salt-loosing nephropathy with polyhydramnios, polyuria and hypokalemic alkalosis. Three renal transporter genes have been identified as responsible. A fourth variant (BSND) [OMIM 602522] is associated with sensorineural deafness and end-stage renal failure. We recently refined the BSND locus between flanking markers D1S2661 and D1S475 (Nephrol Dial Transplant 15:970, 2000). We here performed physical mapping in the BSND critical region and identified a novel gene within a gap of the human genome sequencing projects. By detection of 5 likely loss of function mutations in patients with BSND we identified this novel gene, BSND, as causative. Expression studies demonstrated strong expression in human kidney. In situ hybridization in mouse embryonic kidneys showed a pattern consistent with renal tubular expression on days 14.5 and 18.5 pc. Thus, BSND might represent a novel regulator of transport proteins implicated in aBS, or else, a novel transport protein itself.

Program Nr: 146 from the 2001 ASHG Annual Meeting

Identification of the mouse *cocoa* (*coa*) gene, a candidate gene for human Hermansky-Pudlak syndrome (HPS).

R. Spritz[1], T. Suzuki[1], E. Novak[2], Q. Zhang[2], W. Li[2], E. Sviderskaya[3], S. Hill[3], D. Bennett[3], R. Swank[2]. 1) Hum Med Genet Prog, Univ Colorado Hlth Sci Ctr, Denver, CO; 2) Dept Molec Cell Biol, Roswell Park Cancer Inst, Buffalo, NY; 3) Dept Anat Devel Biol, St. George's Hosp Med Sch, London, UK.

Hermansky-Pudlak syndrome (HPS) is a rare autosomal recessive disorder characterized by tyrosinase-positive oculocutaneous albinism, bleeding tendency, and an ill-defined ceroid-lipofuscin lysosomal storage disease, with frequent fatal complications. We previously identified the *HPS1* gene, which accounts for virtually all cases of HPS in Puerto Rico, but only about half of HPS in non-Puerto Rican patients.

In the mouse, more than 15 loci are associated with mutant phenotypes similar to human HPS, and we previously showed that the mouse homolog to human HPS is *pale ear* (*ep*). Another mouse mutant, *cocoa* (*coa*), has a similar phenotype and is thus a candidate for a minor human HPS locus. Accordingly, we mapped and positionally cloned mouse *coa*, located on chromosome 3, in a region homologous to human chromosome 3. We identified obvious pathological mutations of *coa* in all 4 extant mouse *coa*-mutant alleles. We next characterized the human *coa* gene homolog, and screened for mutations in 19 HPS patients who lacked mutations in the *HPS1* gene, but did not find pathological mutations in any of these patients.

The *coa* gene encodes a novel, ubiquitously-expressed protein of unknown function. Immunofluorescence analysis of melanotic and non-melanotic cells transfected with FLAG-tagged mouse *coa* cDNA showed perinuclear localization in a pattern similar to that of the HPS1 protein. Light and electron microscopy of a permanent line of melanocytes, derived from *coa*-mutant mice, showed markedly hypopigmented melanosomes. We are currently characterizing the precise location of the coa protein and its role in biogenesis of melanosomes and lysosomes.

Program Nr: 147 from the 2001 ASHG Annual Meeting

Consequences of BRCA1 missense mutations in the RING domain may be revealed by the solution structure of the BRCA1/BARD1 complex. *P.S. Brzovic[1], P.L. Welcsh[2], M-C. King[2], R.E. Klevit[1]*. 1) Department of Biochemistry, University of Washington, Seattle, WA; 2) Departments of Medicine and Genomic Sciences, University of Washington, Seattle, WA.

The first BRCA1 protein motif identified was the RING finger domain near the N-terminus. The RING domain of BRCA1 heterodimerizes with BARD1, another N-terminal RING domain protein. The RING domain of BRCA1 has ubiquitin ligase activity in the presence of the ubiquitin transferase UbcH5. This activity dramatically increases when BRCA1 is complexed with the BARD1 RING domain. While the precise mechanism by which loss of BRCA function leads to breast and ovarian cancer remains unknown, it is tempting to postulate that disruption of the BRCA1/BARD1/UbcH5 complex contributes. If the BRCA1/BARD1/UbcH5 complex regulates cellular levels of critical growth factors, then its disruption may lead to an overall increase in steady-state levels of proteins that stimulate proliferation.

Our recently-reported solution structure of the BRCA1/BARD1 RING domain complex (Brzovic et al., submitted) provides a framework for evaluating effects of amino terminal BRCA1 mutations on the BRCA1/BARD1 heterodimer and on its ubiquitin transferase activity. BRCA1 mutations that disrupt critical Zn2+ liganding residues clearly predispose to breast and ovarian cancer. The consequences of other amino acid substitutions in the BRCA1 RING domain are not yet clear. Intriguingly, several of these mutations line the BRCA1/BARD1 dimerization surface. To identify those mutations that affect BRCA1/BARD1 heterodimerization and thereby assess their clinical relevance, we assay the consequences of single amino acid substitutions in BRCA1 using the yeast two hybrid system and quantitative -galactosidase assays. Mutations that destabilize the BRCA1/BARD1 complex or reduce the affinity with which BRCA1 and BARD1 interact are most likely to predispose to disease.

Program Nr: 148 from the 2001 ASHG Annual Meeting

Germline carriers of *ATM* mutations are prevalent among Austrian hereditary breast and ovarian cancer (HBOC) patients. *Y.R. Thorstenson[1], A. Roxas[1], R. Kroiss[2], T. Wagner[2], P.J. Oefner[1].* 1) Stanford Genome Technology Ctr, Palo Alto, CA; 2) Dept Obstetrics and Gynecology, University of Vienna, Vienna, Austria.

ATM is the gene responsible for the inherited disease ataxia-telangiectasia (A-T). The frequency of A-T carriers among women suffering from breast cancer has been estimated to be 6.6%, based on breast cancer incidence in A-T families. Following the successful cloning of *ATM*, several studies have attempted to corroborate this observation and all but one failed to do so. The failure to find *ATM* mutations may be due to the low sensitivity of the detection methods employed. Using DHPLC, which is 96-100% sensitive for detecting sequence variants, we found germline *ATM* mutations in at least 8.7% of 138 Austrian women suffering from breast cancer. The prevalence of ATM mutations in the Austrian HBOC families was similar to that of *BRCA2* and about half of that for *BRCA1*. None of the patients with *ATM* mutations carried either a *BRCA1* or a *BRCA2* mutation. A total of 11 different, functionally detrimental mutations were observed. Three were frameshift or truncation mutations, found in 2.9% of the patients. Three were non-conservative amino acid changes located in the extremely conserved carboxy terminus of the ATM protein, found in 2.9% of patients. One additional conservative and four additional non-conservative amino acid changes were found. One of them, L1420F, had been observed previously in an A-T patient and was found in 2.9% of patients. Using genotype information from first-degree relatives of ten of the carriers in the study, the penetrance was estimated to be 85% at age 60. In conclusion, the risk for breast cancer in A-T carriers appears to be comparable to that for other breast cancer genes.

Program Nr: 149 from the 2001 ASHG Annual Meeting

Genome-wide scanning for linkage in Finnish breast cancer families. *P. Huusko[1, 6], E. Gillanders[1], P. Vahteristo[3], L. Sarantaus[3], S. Juo[2, 5], T. Kainu[1], K. Rapakko[6], M. Jones[1], C. Markey[1], H. Eerola[3], M. Allinen[6], P. Vehmanen[3], J. Leisti[6], G. Blanco[7], C. Blomqvist[4], J. Trent[1], J. Bailey-Wilson[2], R. Winqvist[6], H. Nevanlinna[3], O. Kallioniemi[1]*. 1) Cancer Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Inherited Disease Research Branch, NHGRI, NIH, Bethesda, MD; 3) Department of Gynecology and Obstetrics, Helsinki University Central Hospital, Finland; 4) Department of Oncology, Helsinki University Central Hospital, Finland; 5) Columbia Genome Center, New York, NY; 6) Department of Clinical Genetics, Oulu University Hospital/University of Oulu, Finland; 7) Department of Oncology, Oulu University Hospital/University of Oulu, Finland.

Mutations in the BRCA1 and BRCA2 genes account for a smaller proportion of hereditary breast cancer cases than previously suspected. Less than 20% of Finnish families with 3 or more breast cancers showed BRCA1 or BRCA2 mutations. We recently reported a possible third breast cancer susceptibility locus at 13q21-q22 by linkage analysis in 77 Scandinavian breast cancer families. This new locus explains only about 30% of the remaining families suggesting presence of additional susceptibility loci. Here, we undertook an exploratory genome-wide search focusing on a sample of 14 multiplex breast cancer families from the genetically homogeneous Finnish population. These families tested negative for BRCA1 and BRCA2 and showed no linkage to the distal 13q21-q22 region. Simulations generated by the FASTSLINK program assuming genetic homogeneity predicted an expected maximum LOD score of 1.25 (52% of replications with LOD =1, 21% =2 and 3% = 3). Genome-wide linkage analysis was performed with 398 dinucleotide repeat markers on ABI 377 DNA sequencers. Based on a modified CASH model a peak two-point LOD score of 1.61 (theta=0.0) was seen at 2q. After genotyping additional family members using 34 polymoprhic markers over a 40 cM region, we obtained a peak lod score of 1.99 (theta=0.05) at the chromosomal region 2q31-q33. In conclusion, the results provide preliminary evidence of a novel susceptibility locus for breast cancer at 2q. The results warrant analysis of this and other candidate novel susceptibility loci in other populations.

Program Nr: 150 from the 2001 ASHG Annual Meeting

Identification of putative breast cancer tumour suppressor gene at 16q24.3. *D.F. Callen[1], A. Bais[1], J. Crawford[1], A. Gardner[1], O. McKenzie[1], J. Powell[1], G. Secker[1], C. Settasatian[1], R. Seshadri[2], A-M. Cleton-Jansen[3], C. Cornelisse[3], M. Kotchetkova[1], G. Kremmidiotis[1]*. 1) Dept Cytogenetics/Molec Gen, Women's & Children's Hosp, North Adelaide, South Australia, Australia; 2) Flinders Medical Centre, Bedford Park, South Australia, Australia; 3) Leiden University Medical Centre, Leiden, The Netherlands.

Loss-of-heterozygosity (LOH) studies have identified 16q24.3 as a region that contains a breast cancer tumour suppressor gene. A physical map of this 3Mb region was generated. A single gap in this map, due to the presence of complex repeats, is being closed. Genomic sequence was generated for the majority of the region. This sequence, together with that publicly available, was used to generate a transcript map. Real-time RT-PCR was used to screen for expression differences of these transcripts in a panel of breast cancer cell lines. Targeted genes were screened for mutations in a panel of breast tumours and cell lines showing LOH of the 16q24.3 region. One gene, BNO64, was down regulated in some cell lines. No specific tumour associated mutations have yet been found that disrupt BNO64. Down regulation was due to hyper-methylation of an adjacent CpG island. RNA insitu hybridisation showed that BNO64 was highly expressed in the epithelial cells of the normal mammary duct. Tumours were found that did not express this transcript. Preliminary data suggest that when BNO64 is over expressed in breast cancer cell lines there is loss of normal growth. Further studies are in progress. Confirmation of the role of BNO64 as a breast cancer tumour suppressor is difficult unless specific inactivating mutations are found in tumours.

Program Nr: 151 from the 2001 ASHG Annual Meeting

Targeting epigenetic changes associated with retinoid resistance:a novel strategy to treat breast cancer. *S. Sirchia[1], E. Sironi[1], G. Somenzi[1], R. Pili[2], S. Pozzi[1], N. Sacchi[1,2].* 1) School of Medicine, University of Milan, Milan, Italy; 2) Oncology Center, Johns Hopkins University, Baltimore, MD.

Novel therapeutic approaches are needed in the fight against breast cancer. Vitamin A and its analogs have been tested for prevention and treatment of solid tumors including breast cancer. Unexpectedly, the results have not been as promising as suggested by animal studies. It has become clear that a major hurdle of retinoid- differentiation therapy is represented by the retinoid resistance of human breast cancer cells. Recently, we have identified one cause of retinoid resistance, the silencing of the retinoic acid receptor beta (RAR beta)gene. RAR beta silencing is caused by epigenetic changes, namely DNA methylation and repressive chromatin remodeling of the RAR beta P2 promoter. Fortunately, epigenetic changes can be reversed. We developed a strategy to overcome the silencing at RAR beta by specifically targeting the epigenetic defect at RAR beta P2, using combinations of histone deacetylase inhibitors (HDACIs), a class of chromatin remodeling drugs and RAR beta agonists. Our data show that: 1) in breast cancer cell lines with an unmethylated P2, reacetylation at P2 is achieved by retinoic acid (RA) treatment alone and 2) in breast cancer cell lines with a methylated P2, RAR beta transcription can be restored by combining HDACIs, such as trichostatin A (TSA) and phenyl butyrate (PB), and RA. Thus , histone reacetylation of the chromatin embedding P2 seems to be an absolute requirement for restoring the expression of RAR beta. Apparently, RAR beta transcription can be restored even if P2 remains methylated, suggesting that RAR beta re-expression is possible when P2 undergoes an appropriate chromatin remodeling compatible with transcription. We conclude that: 1) the RAR beta P2 methylation status is a predictor of retinoid response in breast cancer and 2) an appropriate combination of HDACIs and RAR beta agonists can be beneficial to restore RA sensitivity in RA-resistant breast tumors. This study provides the background for clinical trials for human breast cancer. Acknowledgements: This project has been partially funded by the DAMD 17-99-1-9241, DOD-USAMRDC grant to N.S.

Program Nr: 152 from the 2001 ASHG Annual Meeting

Polymorphisms in *BRCA1* and 17b**-hydroxysteroid dehydrogenase 2 (*EDH17B2*) genes as modifiers of ovarian cancer risk in carriers of *BRCA1* germline mutations.** *O.M. Sinilnikova[1,3,6], S. Ginolhac[1,7], S. Gad[2], B. Bressac-de-Paillerets[3], A. Chompret[3], Y-J. Bignon[3], J-P. Peyrat[3], J. Fournier[3], C. Lasset[3], D. Muller[3], J-P. Fricker[3], A. Hardouin[3], P. Berthet[3], M. Longy[3], C. Nogues[3], R. Lidereau[3], C.M. Maugard[3], S. Olschwang[3], C. Toulas[3], R. Guimbaud[3], H.T. Lynch[4], M. Corbex[1], D. Goldgar[1], G.M. Lenoir[1,5], D. Stoppa-Lyonnet[2,3].* 1) Intl Agency Research on Cancer F-Lyon; 2) Institut Curie F-Paris; 3) Groupe Genetique et Cancer FNCLCC, France; 4) Creighton University Omaha, NB; 5) Institut G.Roussy F-Villejuif; 6) Hospices Civils de Lyon; 7) CNRS-UMR 5641 F-Lyon.

Strong heterogeneity of breast and ovarian cancer risk in *BRCA1* carriers suggests that modifying genetic and environmental factors may play a role in risk determining. The goal of this study was to assess if the polymorphisms of the *BRCA1* wild-type allele and the *EDH17B2* gene, coding for one of the key enzymes of oestrogen metabolism, modify the susceptibility to ovarian cancer in the *BRCA1* mutation carriers. These genes appear to be plausible candidates given their functional relevance and a frequent LOH occurrence in the tumours of *BRCA1* carriers of the wild-type chromosome 17q region containing *BRCA1* and *EDH17B2* genes.

Four *BRCA1* and two *EDH17B2* polymorphisms, with population frequency of rare allele ranging from 0.07 to 0.50, were tested in a sample of 460 female *BRCA1* carriers including 119 ovarian cancer cases and 341 ovarian cancer-free individuals, both groups matched for age. In order to conclude on the possible *trans* effect of the polymorphisms of the *BRCA1* wild-type allele it will be necessary to complete in heterozygous individuals to classify the polymorphic variants according to whether they are carried by the wild-type or mutant *BRCA1* allele. The *EDH17B2* polymorphisms Ser313 and TIVS4+50 found to be in strong linkage disequilibrium appeared to have a protective effect against ovarian cancer in the *BRCA1* carriers affected with breast cancer (OR 0.54, 95%CI 0.32-0.91). The same result was obtained when only unrelated cases were included in the analysis. Our finding suggests that certain polymorphic forms of *EDH17B2* could influence the ovarian cancer risk in *BRCA1* carriers.

Program Nr: 153 from the 2001 ASHG Annual Meeting

Polymorphisms in XRCC1 and XPD as Breast Cancer Risk Modifiers in BRCA1 Mutation Carriers. *B. Amirimani[1], S.L. Neuhausen[2], T. Tran[2], T.R. Rebbeck[1], B.L. Weber[1]*. 1) University of Pennsylvania, Philadelphia, PA; 2) University of Utah, Salt Lake City, UT.

BRCA1 accounts for dramatically increase lifetime risk of breast cancer. However, there is variability in the penetrance of breast cancer in BRCA1 mu-tation carrier women. Therefore, we hypothesize that polymorphisms in low penetrance genes, called modifier genes, may play a role in BRCA1-associated breast cancer risk. The role of BRCA1 in DNA damage response suggests that other genes involved in cellular response to DNA damage are plausible candidates for low penetrance modifier genes. In order to evaluate the role of genetic polymorphisms in DNA damage re-sponse genes in BRCA1-associated breast cancer risk, we performed a case-control analysis by genotyping 414 BRCA1 mutation carrier women (208 af-fected with breast cancer and 206 without a breast cancer diagnosis) The genotypes examined include polymorphic sites in XRCC1 (exon 6 Arg194Trp and exon 10 Arg399Gln) involved in base excision repair, and XPD (exon 6 C>A, 156Arg, exon 10 Asp312Asn, exon 22 C>T, Asp711, and exon 23 Lys751Gln), which is active in nucleotide excision repair and transcription cou-pled repair. Three of four genotypes in XPD showed statistically significant association with breast cancer risk: Lys751 in exon 23 (age- and birth cohort-adjusted odds ratio (OR): 1.89; 95% CI: 1.10-3.22), Asp711 in exon 22 (OR: 2.02; CI: 1.11-3.66) and Arg156 in the exon 6 (OR: 3.96; CI: 1.92-816) showed asso-ciation with increased breast cancer risk in BRCA1 mutation carriers. No as-sociation was observed for any of the polymorphisms in the XRCC1 gene studied here. Our results suggest that XPD may be a potential modifier of BRCA1-associated breast cancer. Additional studies will be required to eluci-date the biological basis for this association.

Program Nr: 154 from the 2001 ASHG Annual Meeting

Genetic polymorphisms and survival in a large population-based study of breast cancer. *E.L. Goode[1,2], A.M. Dunning[2], B. Kuschel[2], C.S. Healey[2], F. Durocher[2], J.M. Lipscombe[2], K. Redman[2], B.A.J. Ponder[2], N.E. Day[2], D.F. Easton[2], P.D.P. Pharoah[2] and The Anglian Breast Cancer Study Group.* 1) Fred Hutchinson CA Res Ctr, Seattle, WA, USA; 2) University of Cambridge, Cambridge, UK.

Somatic genetic alterations in tumors are known to correlate with survival, but little is known about the prognostic significance of germline variation. We assessed the effect of germline variation on survival among women with breast cancer participating in the British population-based Anglian Breast Cancer Study. Up to 2,430 cases for whom current vital status data were available were screened for BRCA1/2 mutations and genotyped for polymorphisms in 22 DNA repair, hormone and carcinogen metabolism, and other genes. The effect of genotype on outcome was assessed by Cox regression analysis considering time to death from any cause: 200 deaths occurred during a total time at risk of 6,662 years. The largest effect was observed for the silent polymorphism D501D (t>c) in LIG4, a gene involved in DNA double strand break repair. The estimated hazard ratio (HR) in cc homozygotes relative to tt homozygotes was 4.0 (95%CI 2.1-7.7; p=0.002), and this effect remained after stratification by stage, grade, and tumor type (HR=4.2; 95%CI=1.8-9.4; p=0.01). This finding suggests the presence of a polymorphism at the LIG4 locus, in linkage disequilibrium with D501D, which affects outcome. Total length of a CYP19 intron 4 $[ttta]_n$ repeat was also associated with survival (HR=0.9; 95%CI 0.8-1.0; p=0.01), but this protective effect was non-significant after stratification by stage, grade, and tumor type. Poorer survival was observed for 10 BRCA1 mutation carriers (HR=4.1; 95%CI 1.3-13; p=0.047); however, after adjustment for known prognostic factors, the HR estimate decreased to 2.0 and became non-significant (p=0.4). CYP17 (p=0.05) and TP53 (p=0.06) polymorphisms showed marginally significant associations in unstratified analyses, however no effect on survival was seen for polymorphisms in ATM, BRCA1/2, CHK2, KU70, NBS1, RAD51, RAD52, XRCC3, AR, COMT, NQO1, VDR, ADH3, CYP1A1, GSTP1, TGFB, or CDH1. These results may provide insight into the biological determinants of response to treatment and prognosis in breast cancer.

Program Nr: 155 from the 2001 ASHG Annual Meeting

Dietary patterns and genetic susceptibility to lung cancer risk. *Y.-Y. Tsai[1], K.A. McGlynn[1], A.B. Cassidy[2], Y. Hu[1], J. Arnold[2], P.F. Engstrom[2], K.H. Buetow[1]*. 1) DCEG/NCI, Bethesda, MD; 2) Fox Chase Cancer Center, Philadelphia, PA.

Polymorphisms in genes encoding enzymes involved in activation or detoxification for smoking-related carcinogens have been associated with variability of lung cancer susceptibility. Certain dietary constituents can act as substrates or inducing agents for these enzymes and influence carcinogen metabolisms. Due to the complexity of carcinogenesis and intercorrelations between dietary constituents, we used dietary patterns generated from cluster analysis and examined the association between 15 variants of genes involved in the phase II metabolism and lung cancer risk. A total of 438 subjects (254 cases and 184 controls) were recruited. Cluster analysis was performed using the nutrient densities of the four major dietary constituents: protein, carbohydrate, animal fat, and dietary fiber, to classify clusters of persons with similar intakes of these components. Two groups of individuals with distinct dietary patterns were identified: (1) the unhealthy diet group: 151 cases and 90 controls with high intake of animal fat and protein, but low intake of carbohydrate and dietary fiber; (2) the healthy diet group: 103 cases and 94 controls who consumed high amount of fibers and carbohydrate but low amount of protein and animal fat. Results of logistic regression have shown that subjects with a healthy dietary pattern are at a lower lung cancer risk (0.653; 95%CI:0.446-0.957), but this effect disappeared after adjusting for smoking. On stratified analysis of 329 smokers, a healthy dietary pattern is associated with decreased lung cancer risk among the GSTM1-null individuals (0.466; p=0.018), after adjusting for sex and age. In the unhealthy diet group, smokers with a variant genotype on exon3 of EPHX1 have a higher lung cancer risk, compared to those with homozygosity of Tyr-113 alleles (2.173; p=0.039). These results suggest that dietary factors can influence the role of certain genes encoding metabolic enzymes in lung cancer risk. Without clarification of complex correlations between dietary constituents, adjustments using dietary patterns may be useful to elucidating the independent effect of polymorphisms in genes responsible for carcinogen metabolisms.

Program Nr: 156 from the 2001 ASHG Annual Meeting

***Drosophila* homologue of the human Multiple Endocrine Neoplasia Type I gene (MEN1) interacts with Jun and Fos *in vivo*.** *K. Suphapeetiporn, V. Busygina, T. Xu, A.E. Bale.* Yale University School of Medicine, New Haven, CT.

Analysis of the genes responsible for hereditary cancer predisposition and the pathways in which they act has been critically important in providing insight into the normal regulation of cell growth and differentiation. MEN1 is an autosomal dominant cancer syndrome characterized by pituitary, parathyroid, pancreatic islet and carcinoid tumors. The function of the MEN1 gene product is unknown, but biochemical studies have shown an interaction between MEN1 and six other proteins including the JunD transcription factor (Agarwal et al., 1999). The biological significance of these interactions is not established. Sequencing of the Drosophila genome identified a homologue of MEN1 with 46% identity to the human protein. We used the UAS-GAL4 system to overexpress Drosophila MEN1 (DMen1) in tissue specific patterns. Broad overexpression of DMen1 under the control of an actin promoter resulted in early embryonic lethality, and DMen1 expressed in the ectoderm under the control of the 69B-Gal4 promoter caused pupal lethality. Restriction of overexpression to dorsal ectodermal tissues (pannier promoter) resulted in viable flies with a thoracic cleft. This phenotype is similar to that caused by reducing the activity of DJun. Genetic interaction studies revealed that the DMen1 thoracic cleft phenotype could be completely suppressed by overexpression of wild type DJun. In mammals, as well as in Drosophila, Jun is known to heterodimerize with Fos to form an AP-1 transcription complex that controls the expression of multiple target genes. We found that overexpression of dominant-negative DFos enhanced the DMen1 thoracic cleft phenotype, and paradoxically, wild type DFos had the same effect. Our data are consistent with a model in which MEN1 acts as a corepressor of Jun transcriptional activity. These findings also suggest that decreasing the activity of AP-1 or changing the balance of Jun/Fos activity in favor of Fos results in dysregulation of downstream target genes and the thoracic cleft phenotype.

Program Nr: 157 from the 2001 ASHG Annual Meeting

Defects associated with anorectal atresia/stenosis: A population-based study. *S.A. Rasmussen, C.A. Moore, R. Trimpert, D. Gambrell.* Centers for Disease Control and Prevention, Atlanta, GA.

Anorectal atresia/stenosis is a relatively common congenital defect, but little is known about its etiology. Most studies of this defect have been hospital-based, potentially introducing bias. For this study, we used population-based data from the Metropolitan Atlanta Congenital Defects Program, an ongoing birth defects surveillance program with active case ascertainment methods, for 1968 through 1997. We identified 364 infants with anorectal atresia/stenosis among 937,195 births, for a birth prevalence of 1 in 2,575. Males were observed more often than females (M:F = 1.4:1); sex was unknown for 16 (4.4%) because of ambiguous genitalia. Two infants (an acardiac twin and a conjoined twin) were excluded from further analysis. Of the remaining 362 infants, 19 (5%) had syndromes, 205 (57%) had additional major defects, and 138 (38%) had isolated anorectal atresia/stenosis. The most commonly observed syndromes were Trisomy 21, Trisomy 18, and Trisomy 22. Defects most frequently observed among the nonsyndromic infants involved the renal/urinary tract (28%), genital/reproductive tract (23%), and cardiovascular system (16%). Three or more VATERL association anomalies were observed in 39 (11%) infants. Forty-four infants with anorectal atresia/stenosis (one isolated, 41 with additional major defects, and two with syndromes) were noted to have a single umbilical artery. Infants with a single umbilical artery were 1.8 times more likely (95% CI = 1.6-2.0) to have additional major defects than infants with normal umbilical arteries. Most infants with anorectal atresia/stenosis and single umbilical artery had additional major defects involving the caudal body trunk (e.g., sirenomelia, exstrophy, and urogenital defects). Abnormal development of the caudal body structures has previously been associated with single umbilical (vitelline) artery, hypothesized to be secondary to nutritional deficiency resulting from vascular steal via the persistent vitelline artery. These findings may guide clinicians in their evaluations of infants with this defect and provide important insights into mechanisms of anorectal atresia/stenosis.

Program Nr: 158 from the 2001 ASHG Annual Meeting

A 1-year prospective study of the incidence of the 22q11.2 molecular deletion in 303 fetuses with heart defects. *R. Berry[1], J. Horwitz[1], S. Bhatt[2], M. Shaham[3], R. Miller[4], J. Moore[1]*. 1) Genzyme Genetics, Santa Fe, NM; 2) Orange, CA; 3) Yonkers, NY; 4) Tampa FL.

It has been reported that 12.5% of children with congenital heart defects (CHD) have deletions of 22q11.2 (SB Olsen et al. Am J Hum Genet 55:A975, 1994). This deletion is often found in patients with DiGeorge/Velocardiofacial syndrome and has been associated with a wide variety of cardiac defects, the most common defect being the conotruncal type. However, studies of prenatally diagnosed CHD and 22q11.2 deletion have been limited. One large 6-year prospective study of 261 fetuses with conotruncal cardiac defects found that 20.7% had a 22q.11.2 deletion (Y Boudjemline et al. J Ped 138: 520-524, 2001). A retrospective analysis of prenatal specimens received at our laboratory over an 8-month period with a heart defect, normal karyotype, and FISH studies ordered by the physician, identified 8 of 105 (7.6%) with the deletion 22q11.2. Subsequently, a 1-year prospective study was conducted. From April, 2000 - April, 2001, we proactively offered 22q11.2 deletion testing for all prenatal specimens with an indication of fetal heart defect (excluding echogenic foci) and with a normal karyotype. Of 303 specimens which fit these criteria, and for which the physician authorized the testing, 11 (3.6%) were identified to have a deletion of 22q11.2. In fetuses with the deletion, the cardiac abnormalities detected by ultrasound included truncus arteriosus, aortic stenosis, VSD (3), tetralogy of Fallot (2), malrotation of the heart, and cardiac hypertrophy, as well as 2 unspecified heart defects. Although the majority of these abnormalities can be classified as conotruncal defects, several were not specifically identified as such. One of the positive cases in our retrospective study had echogenic foci as the only reported cardiac finding on ultrasound. Our study indicates that FISH studies for the 22q11.2 deletion are appropriate for fetuses with an ultrasound finding of a fetal cardiac defect and a normal prenatal karyotype.

Program Nr: 159 from the 2001 ASHG Annual Meeting

The effect of prenatal or postnatal surgery for spina bifida on maternal adjustment. *E. Peach[1], J. Bruner[2], S. Oppenheimer[1], G. Yager[1], J. Bean[1], N. Warren[1]*. 1) Univ.of Cincinnati, Cincinnati,OH; 2) VUMC, Div.of Maternal/Fetal Medicine, Nashville,TN.

Fetal,or prenatal,surgery for spina bifida is an experimental alternative to the traditional spinal repair following birth. Prenatal repair of the spinal cord is performed while the child is still in the womb, placing both the mother and fetus at risk for complications due to prematurity and Caesarian section. Short-term outcome studies suggest children who have had the prenatal repair have a decrease in hindbrain herniation and a decreased need for hydrocephalus shunts compared to children who had postnatal repair. Thus far,long-term neurological benefits of prenatal repair remain unknown. Additionally, there have been no studies regarding the effects of prenatal repair on maternal psychological status. This study compares the psychological adjustment of mothers whose children had prenatal repair to that of mothers whose children had postnatal repair. A self-administered questionnaire was mailed to mothers who gave birth to children with spina bifida between April 1997 and November 2000. The participants included 200 mothers whose children had postnatal repair and are followed at seven Spina Bifida Clinics and 60 mothers whose children had prenatal repair at Vanderbilt University Medical Center. The questionnaire was composed of four parts:Maternal Adjustment and Maternal Attitudes(MAMA) questionnaire,Questionnaire on Resources and StressShort Form(QRS-SF),a support measure,and patient demographics. The study revealed slightly higher maternal adjustment levels in the prenatal repair cohort(mean=63) compared to the postnatal repair group(mean=61),although not significant. The prenatal cohort had significantly lower levels of stress($p=0.0096$) and higher levels of support in 4 out of 6 areas:spouse($p=0.0124$), friends($p=0.0133$),family($p=0.0333$),and Spina Bifida Associations($p<.0001$), with only health professionals and religion not reaching significance. These results reinforce the need for genetic professionals to explore aspects of maternal psychological status,support,and stress levels with families when presenting prenatal and postnatal repair options for spina bifida.

Program Nr: 160 from the 2001 ASHG Annual Meeting

Maternal serum ITA utility for prenatal Down Syndrome detection: A pilot study using a new automated assay.

J.E.S. Lee[1], L.A. Cole[2], G.E. Palomaki[3], M.J. Mahoney[4], P. Benn[5], T. Vendely[1], C.M. Strom[1], R. Pandian[1]. 1) Quest Diagnostics' Nichols Inst, San Juan Capistrano, CA; 2) Univ New Mexico, Albuquerque, NM; 3) Fdn for Blood Research, Scarborough, ME; 4) Yale Univ, New Haven, CT; 5) Univ Conn, Farmington, CT.

Background: Invasive trophoblastic antigen(ITA) is a hyperglycosylated form of hCG. It has been reported to be significantly elevated in second trimester serum and urine from women carrying a fetus affected with Down syndrome (DS).

Methods: Using an automated immunochemiluminometric assay for ITA (Nichols Institute Diagnostics), we analyzed second trimester(15-21 weeks gestation) serum from 15 DS and 82 gestational age-matched controls that had been stored at -60°C for 3 years. AFP was measured by the Abbott Axsym Tumor Markers AFP method.

Results: Serum ITA values in control pregnancies decreased an average of 19% per week. Smoothed median values at 15 and 20 weeks were 31.0 and 10.7 ng/mL, respectively. All results were converted to multiples of the gestational age-specific median (MoM). The DS pregnancies had a median ITA level of 4.99 MoM (range 1.45 to 15.3 MoM). Overall, 11 of 15 (73%) DS cases were above the 95th centile (3.27 MoM). When combined with maternal age, detection increased to 81% at a 5% false positive rate (FPR). Detection remained at 81% when the FPR was reduced to 3%. Adding AFP measurements further increased the detection rate to 87%. For comparison, the most effective combination of markers currently in use (AFP, uE3, hCG, & dimeric inhibin A) can detect 77% of DS cases at a 5% FPR.

Conclusions: Second trimester serum ITA levels are about 5 times higher in DS pregnancies than in unaffected pregnancies. ITA is the most discriminating marker reported to date. When combined with maternal age and AFP measurements, performance appears to be better than the current "Quad" screen. These preliminary observations warrant confirmation in a larger data set.

Program Nr: 161 from the 2001 ASHG Annual Meeting

Fetal gender and aneuploidy detection using fetal cells in maternal blood: analysis of NIFTY 1 data. *L. Jackson[1], D. Bianchi[2], J. Simpson[3], S. Elias[4], W. Holzgreve[5], M. Evans[6], K. Dukes[7], L. Sullivan[7], K. Klinger[8], F. Bischoff[3], S. Hahn[5], K. Johnson[2], D. Lewis[3], R. Wapner[6], F. Dela Cruz[9] and NICHD Fetal Cell Isolation Study Group.* 1) Dept Medicine, TJU, Phila PA; 2) Tufts-NEMC, Boston MA; 3) Baylor Coll Med, Houston TX; 4) UIC, Chicago IL; 5) Univ Basel, Basel,Switzerland; 6) MCP-Hahnemann, Philadelphia, PA; 7) DM-Stat, Medford MA; 8) GENZYME, Boston, MA; 9) NICHD, Bethesda, MD.

The National Institute of Child Health and Human Development (NICHD) Fetal Cell Isolation Study (NIFTY) is a prospective multi-center clinical project to develop non-invasive methods of prenatal diagnosis. The initial objective was to assess the utility of fetal cells in the peripheral blood of pregnant women to diagnose or screen for fetal chromosome abnormalities. Results of fluorescence in situ hybridization (FISH) analysis of interphase nuclei of these cells were compared to metaphase karyotypes of fetal cells obtained by amniocentesis or chorionic villus sampling (CVS). We report the analysis of the data from 2,747 fully processed pre-procedural blood samples, 1,289 from women carrying singleton male fetuses. Following initial cell separation by density gradient centrifugation, target cell recovery and fetal cell detection were better using magnetic-based separation systems (MACS) than with flow-sorting (FACS). Blinded FISH assessment of samples obtained from women carrying singleton male fetuses found at least one cell with an X and Y signal in 41.4% (95% CI: 37.4%, 45.5%). The comparable blinded detection rate for finding at least one aneuploid cell was 36.4%, while in non-blinded samples it was 86.7%. Combining blinded and non-blinded cases, the overall detection of aneuploidy was 73.2%, which is comparable to single marker antenatal serum screening. Smaller study sample cohorts found blinded X/Y detection rates over 70% with false positive rates below 1% suggesting that technological modifications are needed before fetal cell analysis will find clinical application in noninvasive prenatal screening or diagnosis. Promising modifications from the current study are under development in an ongoing study (NIFTY II).

Program Nr: 162 from the 2001 ASHG Annual Meeting

Large amounts of cell-free fetal DNA are present in amniotic fluid. *D.W. Bianchi[1], E.S. Le Shane[1], U. Tantravahi[2], J.M. Cowan[1]*. 1) Division of Genetics, Depts of Peds and Ob/Gyn, Tufts Univ School of Medicine and New England Medical Center, Boston, MA; 2) Dept. of Pathology, Women and Infants' Hospital, Providence, RI.

Fetal cell-free DNA sequences are in the plasma and serum of pregnant women. To better understand the origin and metabolism of these sequences, we analyzed amniotic fluid (AF) samples. We hypothesized that the source of fetal DNA in maternal plasma and serum might be fetal DNA in AF.

Thirty-eight frozen coded AF supernatant samples were thawed and spun to remove rare remaining cells. Cell-free DNA was extracted and amplified via real-time PCR using *FCY*, a Y chromosome-specific sequence, and b globin as a measure of total DNA present.

The mean gestational age of the AF samples was 16.8 weeks (range 14.4-20 wks). In AF from female fetuses, no *FCY* sequences were detected. The mean amount of male DNA detected in male fetuses was 2,668 genome equivalents (GE)/mL amniotic fluid (range 228-12,663 GE/mL). The predicted fetal gender was correct in all cases ($p<0.0001$, Fisher's exact test). In 2 male cases of trisomy 21 fetal DNA in AF was not elevated compared to normal male fetuses. This contrasts with prior reports demonstrating a 2 fold elevation of fetal DNA in maternal plasma when the fetus has trisomy 21.

Significant amounts (100-200X more than in the equivalent volume of plasma) of cell-free fetal DNA are present in AF. The source of the DNA could be fetal kidneys, lung, skin, placenta, or membranes. Cell-free DNA in AF may provide insights into fetomaternal trafficking of nucleic acids.

Program Nr: 163 from the 2001 ASHG Annual Meeting

Direct testing for segregation of chromosomes 13,16, 18, 21 and 22 in human female meiosis. *A. Kuliev, J. Cieslak, Y. Verlinsky.* Dept Molecular Genetics, Reproductive Genetics Inst, Chicago, IL.

It is suggested that chromosomal abnormalities are mainly of meiosis I origin, although no direct observations are available. We introduced direct testing of the outcomes of the first and second meiotic divisions by the first and second polar body FISH analysis, to investigate the origin of aneuploidies in maternal meiosis. Both polar bodies were removed simultaneously following maturation and fertilization of oocytes from 289 IVF patients of over 35, and tested using specific FISH probes for chromosomes 13,16, 18, 21 and 22 (Vysis). FISH results were available in 2169 oocytes, of which 77.3% were with both meiosis I and meiosis II, 11.15% with only meiosis I and 11.6% with only meiosis II data. 1362 (62.8%) oocytes were with segregation errors, of which 518 (38%) resulted from meiosis I, 412 (30.3%) from meisos II and 432 (31.7%) from both. Specific error rates for chromosomes 13,16, 18, 21 and 22 were 8.1% (a total of 110 errors), 8.7% (118), 12.4% (169), 7.8% (106) and 18.2% (248), respectively. In contrast to the expected predominant meiosis I origin of chromosome 16 and 21 errors, and predominant meiosis II origin of chromosome 18 errors, our data showed no significant difference in the origin of chromosome 21 errors, and the opposite tendency for chromosome 16 and 18 errors. The majority of chromosome 16 errors were observed in meiosis II, and the majority of chromosome 18 errors in meiosis I. Chromosome 22 errors equally originated from both meiotic divisions, while chromosome 13 errors predominantly of meiosis I origin, in agreement with DNA polymorphism data. The results demonstrate the clinical significance of testing for both the first and second meiotic division errors in pre-selection of aneuploidy-free oocytes in IVF patients of advanced maternal age.

Program Nr: 164 from the 2001 ASHG Annual Meeting

IVF increases sex chromosome nondisjunction in a murine model system. *C. Bean, T. Hassold, L. Judis, P. Hunt.* Dept Genetics, CWRU, Cleveland, OH.

In vitro fertilization (IVF) is an increasingly important tool for the treatment of human infertility, but there are still concerns about the genetic quality of IVF-derived embryos. For example, several groups have reported increased rates of aneuploidy in pregnancies initiated through intracytoplasmic sperm injection (ICSI) and, in studies of blastocysts derived from "spare" preimplantation embryos, chromosome mosaicism is a common finding. However, because of the difficulties associated with research on human embryos, it has not been possible to ask whether the in vitro procedures themselves are responsible for these abnormalities.

We have been studying an unusual mouse the BALB/cWt ("Wt") male in which the Y chromosome is susceptible to high rates of nondisjunction at the earliest cleavage divisions: by the 8-cell stage, nearly 50% of all Wt Y-carrying embryos are sex chromosome aneuploidy mosaics. As these are the same cleavage divisions that human IVF-derived embryos must complete in an artificial setting, analysis of the Wt Y chromosome provides an opportunity to directly assess the effect of IVF on chromosome segregation.

In initial studies, we performed standard mouse IVF, cultured embryos in 5% CO2 in air and examined the sex chromosome constitutions of 149 Wt Y bearing embryos. FISH analysis of 2, 4, 8 and 16 cell stage embryos showed a significant increase in mosaic sex chromosome aneuploidy at each stage, indicating that IVF increases the risk of aneuploidy. To assess whether culture conditions affect segregation, we repeated the IVF procedure in a lowered oxygen atmosphere and examined the sex chromosome constitutions of 185 Wt Y bearing embryos. Under these conditions the observed mosaicism was reduced to control (in vivo) levels.

Thus, our results suggest that human embryos derived by IVF may be at an increased risk of aneuploidy, due to early cleavage division nondisjunction. However, they also provide optimism that, by altering culture conditions, this increase in risk may be reduced or eliminated.

Program Nr: 165 from the 2001 ASHG Annual Meeting

Follow-up of 30 children with de novo balanced rearrangements and supernumerary marker chromosomes diagnosed at amniocentesis. *A. Gyejye, K. Anyane-Yeboa, D. Warburton.* Columbia University, New York, NY.

Limited data are available concerning the prognosis for fetuses diagnosed prenatally with either an apparently balanced de novo chromosome rearrangement or a de novo supernumerary marker chromosome. In particular, previous risk estimates involved follow-up beyond 2 years of age in only a minority of cases. Parents are often anxious to know whether those infants appearing to be normal in infancy may develop problems later in life. We contacted 39 previously unreported patients seen for genetic counseling because of a diagnosis of a de novo rearrangement or a marker chromosome, and obtained information on health and development in 30 children by phone interview, questionnaire, and /or examination by a clinical geneticist. Age at follow-up ranged from 7 months to 10 years, with 23 children being at least 2 years old.

Of 18 cases where a marker was identified, 7 (38%) chose to terminate the pregnancy. Significant developmental problems were identified in 4 of the 11 remaining children (44%), two with markers not further identified, one with a marker derived from chromosome 14/22 negative for the cat-eye region, and one with an invdup (15) found not to contain SNRPN by FISH. Problems were evident from birth, except for one child where myoclonic jerks developed at 7 months and speech delay is now present at age 4. Of 21 cases with de novo balanced rearrangements, 2 pregnancies were terminated. Two of the 19 livebirths showed developmental abnormalities. One had congenital kyphosis, and is otherwise developing normally at age 2. The other had transposition of the great vessels, and shows speech delay at age 4. Two other children have developed ADHD at 2 and 3 years, but are otherwise reaching normal milestones. These data are consistent with previous data showing a low incidence of severe developmental problems in children with de novo balanced rearrangements and a higher rate of abnormalities in children with supernumerary marker chromosomes. Follow-up beyond infancy has not revealed major problems not identified at birth.

Program Nr: 166 from the 2001 ASHG Annual Meeting

Risk of Mosaicism and Uniparental Disomy associated with the prenatal diagnosis of non homologous Robertsonian translocation carrier. *H. Bruyere[1], G. Robertson[2], R.D. Wilson[1], S. Langlois[1].* 1) Depts. of Pathology, Obstetrics and Gynecology and Medical Genetics, University of British Columbia, Vancouver, Canada; 2) Veracel Inc, Toronto, Ontario, Canada.

Through abnormal segregation at meiosis resulting in a trisomic zygote, and the subsequent loss of one of the 3 copies of the chromosome involved (trisomic rescue), carriers of non-homologous Robertsonian translocations (RT) are at increased risk of mosaicism and uniparental disomy (UPD). In our centre, UPD testing in at-risk prenatal cases has been routinely offered since 1996. This study is a retrospective analysis of our experience and a review of the literature to determine the risk of UPD associated with Robertsonian translocations. Forty four prenatal cases, for which UPD 14 and/or 15 studies were carried out because of a non-homologous RT familial history or diagnosis, were reviewed. UPD 14 (40 cases) and 15 (7 cases) were ruled out in all cases. Taken together with reported cases, UPD studies have been done in 281 prenatal cases because of a history of a RT in the fetus or the parent. In some cases, UPD for both chromosomes involved in the RT was looked for resulting in a total of 449 tests. One case was positive for UPD 13 in addition to being positive for trisomy mosaicism. Using the exact binomial distribution, the overall incidence of UPD in the population tested is between 0.05% and 1%. In the same population, 3 cases of trisomy mosaicism were found (giving a low estimation of incidence between 0.4 and 3%). We conclude that: 1. Fetuses carrying a Robertsonian translocation are at a small risk of UPD (0.05-1%). This risk may not justify a second amniocentesis in cases where not enough cells are available for UPD testing after the cytogenetics analysis is complete. 2. Only in our study were fetuses with a normal karyotype (but RT carrier parent) tested for UPD. Theoretically these fetuses are also at increased risk for UPD and therefore should be tested if sufficient material is available. 3. In this population of fetuses, trisomy mosaicism was more frequent than UPD. This finding would justify the study of additional colonies in all cases of prenatally diagnosed RT.

Program Nr: 167 from the 2001 ASHG Annual Meeting

Pharmacogenetics:the ethical issues. *S.M. Thomas.* Nuffield Council on Bioethics, London , U.K.

Over the past decade, there has been wide discussion of the ethical issues raised by genetic testing and screening. Guidelines for clinical practice have since become widely established.These have been largely based on the identification of single genes for rare diseases. During the next 5-10 years, the guidlelines will need to be expanded to include susceptibility gene testing and pharmacogenetic testing.

In broad terms, several of the ethical and policy issues posed by single gene testing will be relevant to the development and application of pharmacogenetics. Established principles concerning consent, confidentiality, pre-natal testing, testing of children and research procedures will apply. However, there are several spects of pharmacogenetics, which aims to tailor drug treatment to the individual genotype of the patient, which will require careful consideration. This presentation focuses on 3 key questions posed by the pharmacogenetic approach to drug discovery:

- clinical trials will require patient stratification. Should high risk patients identified in research be informed?
- how should non-responders in research who may receive greater exposure to the drug be protected?
- what is a company's obligation to apply its pharmacogenetic knowledge when it is uneconomic to do so?

The pharmacogenetic approach offers the prospect of a substantial reduction in the frequency of adverse events. While it will be argued that it would be unethical not to test patients and risk possible harmful exposure to drugs, clear guidelines are needed for research participants, particularly in relation to what patients are told. This is particularly important for health insurance: high risk patients could be burdened with expensive premiums if their genetic profile implies a high cost drug requirement or makes serious side effects likely.

Program Nr: 168 from the 2001 ASHG Annual Meeting

Variations in Informed Consent Practices for Genetic Research. *S.C. Hull, H. Gooding, E. Warshauer-Baker, S. Metosky, E. Hurley, E. Gutter, B. Wilfond.* Bioethics Research Section, Medical Genetics Branch, NHGRI/NIH, Bethesda, MD.

Over the last 6 years, various recommendations have emerged regarding informed consent for the collection, storage, and use of tissue samples in research. However, there are little data on what information investigators and IRBs provide to subjects. This study describes the content of National Institutes of Health (NIH) intramural consent forms for genetic research. Of the 832 intramural studies approved by one of 14 NIH IRBs in 2000, we evaluated the 258 whose major (n=139) or minor (n=119) purposes involved genetics research and/or collection of tissues for future research. Purposes were considered major if described in the title or first paragraph of the consent form. A 50-item evaluation tool was used to describe the following 6 domains within consent forms: sharing of genetic results, risks, confidentiality, ownership/commercial use of samples, storage of samples, and handling of samples and data upon withdrawal from study. We noted whether consent forms contained a discussion of each of these domains, and the content within each domain. The domains were discussed by varying proportions of consent forms: storage of samples (73%), confidentiality (69%), sharing of genetic results (67%), risks (62%), handling of samples and data upon withdrawal (28%), and ownership/commercial use of samples (16%). Seven percent of forms mentioned all six domains, 17% included 5 domains, 23% included 4 domains, 17% included 3 domains, 12% included 2 domains, 16% included 1 domain, and 7 % discussed none of these domains. Comprehensiveness, as measured by the mean number of domains, varied according to the purpose of the study. Forms whose major purpose was genetic research and/or collection of tissues for future research were more comprehensive (mean=3.7) compared to forms in which these were minor purposes (mean=1.9). A limitation of this study is that the content of consent forms do not necessarily reflect the consent process. This data sets the stage for further discussion about what information subjects consider important in making decisions and what information they ought to be provided.

Program Nr: 169 from the 2001 ASHG Annual Meeting

Family influences on interest and decisions regarding BRCA testing. *T.M. Powell, D.R Cox.* Genetics Dept, Stanford University, Stanford, CA.

It is unclear why there is a large discrepancy between high interest in BRCA testing and actual use among women at genetic risk. This study aims to identify family response patterns that may affect how an individual perceives and acts upon BRCA testing. Having women's perspectives will help define strategies for clinical intervention. One qualitative interview was conducted with each participant using open-ended questions to explore family cancer experiences and attitudes towards breast cancer and BRCA testing in a sample of 53 adult women. These women were predominantly Caucasian and well-educated. Participants were divided into three groups depending on the number of breast and ovarian cancer cases in their family. The interviews were taped and transcribed and then analyzed using grounded theory. There are different levels of interest and non-interest in BRCA testing in women with personal or family history of breast and/or ovarian cancer, regardless of the frequency of cancer cases in that family. Women with a family history of these cancers manage their genetic cancer risk by going through a process which includes developing their risk perception and using various coping mechanisms. Also, a change in family dynamic is occurring simultaneously: individuals develop protective beliefs regarding breast cancer issues, those beliefs become family beliefs, and then family beliefs are passed from one generation to the next. The most important motivation against BRCA testing is the belief that such testing is not useful for reducing breast cancer risk or preventing the disease. In contrast to existing literature which shows that health care professionals perceive BRCA testing as being ultimately useful, the results of this study indicate that those at genetic risk are more concerned about eliminating anxiety produced by having a family history of breast cancer than they are about preventing the disease itself. Individual and family protective beliefs are deeply ingrained in a person's breast cancer risk perception. Understanding at-risk individuals and families' perspective on BRCA testing can improve doctor-patient communication and guide public policy issues in informed consent and genetic education.

Program Nr: 170 from the 2001 ASHG Annual Meeting

Genetic cancer risk assessment (GCRA) at breast cancer (BC) diagnosis. *J.N. Weitzel, K.R. Blazer, J.J. Choi, R.A. Nedelcu, L.E. McCahill, D.J. MacDonald.* Clinical Cancer Genetics, City of Hope Cancer Center, Duarte, CA.

The lifetime risk of BC associated with inherited *BRCA* gene mutations is 50-80%. Early-onset, typical of hereditary disease, is associated with an increased risk for additional primary BCs. Contralateral BC risk is up to 65% in *BRCA* mutation carriers. Although breast conservation therapy (BCT) may be efficacious at 5 years, the incidence of late ipsilateral breast tumor recurrence is 22%-48% at 10 years in hereditary BC. Consequently, some *BRCA* carriers would choose bilateral mastectomy (BM) rather than BCT. **Objective:** We sought to determine the need, feasibility and impact on management decisions of integrating GCRA at the time of BC diagnosis. **Methods:** As part of a prospective study we identified 143 patients affected with BC from 223 women referred to the *Cancer Screening & Prevention Program* for GCRA in the past year. The timing of consultation and the impact on therapeutic decisions was analyzed. **Results:** Among those affected with BC, 25% (37/143) presented for GCRA in the midst of diagnosis and treatment (range 3 to 60 days after diagnosis), and are the focus here. Their median age was 43 years (range 27-62), and the mean calculated probability of a detectable *BRCA* mutation was 23% across the cohort. A mutation was found in 23% (7/31) of those tested, demonstrating the accuracy of the estimation tools. Three women deferred testing because of intercurrent psychosocial stressors, two did not choose testing because of a low probability of mutation and one result is pending. Of the 7 *BRCA* mutation carriers, 2 had undergone BCT for previous BC. Both of these patients chose BM and expressed regret that they had not undergone GCRA at their first diagnoses, since they might have avoided a second BC by choosing this procedure then. The remaining 5 patients with *BRCA* mutations also proceeded with BM. Two patients without mutations proceeded with BM due to persistent fear. The remaining 20 patients who tested negative were reassured and completed stage appropriate treatment. **Conclusions:** GCRA influenced surgical decisions in a risk-appropriate way in the majority of cases, and should be considered for any woman with >10% mutation probability.

Program Nr: 171 from the 2001 ASHG Annual Meeting

Screening for a genetic susceptibility to colorectal cancer. *P.T. Rowley, S. Loader, J. Levenkron, C. Shields.* Div Genetics, Univ Rochester Medical Ctr, Rochester, NY.

Do colon cancer patients identified at increased genetic risk through a cancer registry accept the offer of a genetic evaluation? Of 974 patients diagnosed with colorectal cancer at £60 y from l987-1999 in a 5-county area including Rochester, the physicians of 631 patients (65%) forwarded a cancer family history survey to their patient; 460 (73%) completed the survey. Of these 460, 161 (35%) reported having at least one first- or second-degree relative with colon cancer and were sent a set of questionnaires and a more detailed family cancer history form. Of the 121 who responded, 106 qualified by family history (23% of 460). Of the 95 contacted to date, 45 (47%) have come for a free evaluation. Individuals were more likely to accept evaluation if they had children ($p<.001$), identified a larger number of supportive relatives ($p<.02$), and had more total cancers in the family ($p<.03$). Of the 45 counseled, 31 (69%) chose to have DNA testing at no cost. Of the 95, an individual was more likely to choose DNA testing if the family had a larger number of total cancers ($p<.001$) or colon cancers ($p<.005$), self-rated general health was better ($p<.001$), and Beck depression score was lower ($p<.002$). Of the 30 patients with completed results, 6 (20%) are abnormal. Two abnormalities, K618A (MLH1) and A636P (MSH2) are known to be deleterious, whereas four are of unknown clinical significance, G162R and L911R (both MSH2) in one person each and H718Y (MLH1) in two unrelated persons. In summary, (1) in patients with colorectal cancer at a younger than average age, nearly 1 in 4 reported colorectal cancer in a close relative. (2) When offered a genetic evaluation, nearly half of those qualifying accepted. (3) Following nondirective education about DNA testing, over 70% requested testing. (4) Of patients whose results have been received, 1 in 5 are abnormal. (5) Acceptance of genetic services was related to the magnitude of the threat (no. of colon cancers and total cancers in the family), perceived ability to deal with the threat (low depression, a supportive family, and good health), and a desire to gain information for the sake of their relatives. (CDC UR6/CCU217194).

Program Nr: 172 from the 2001 ASHG Annual Meeting

Clinical Penetrance of Hereditary Hemochromatosis (HH) is Extremely Low. *E. Beutler[1], V. Felitti[2], T. Gelbart[1], N.J. Ho[1], J. Waalen[1]*. 1) The Scripps Research Institute, La Jolla, CA; 2) Kaiser Permanente, San Diego, CA.

Hereditary hemochromatosis (HH) caused by mutations of the HFE gene has often been considered an ideal disorder for genetic screening. The homozygous state occurs in approximately 5/1000 northern Europeans; the disease is readily treated by phlebotomy. Screening for HH has been deemed cost effective, but without knowledge of the penetrance of the homozygous genotype: estimates have been made by examining patient populations or relatives without comparison to suitable controls. We genotyped for the C282Y and H63D HFE mutations 38,732 patients attending the Kaiser Health Appraisal Clinic in San Diego. This analysis is based upon the 33,794 reporting white (including Hispanic) ancestry. 145 (70 M, av age 55.3; 75 F av age 57.5) of these were homozygotes for the C282Y mutation. 92 (57 M, av age 54.1; 35 F av age 60.5) had a transferrin saturation (TS) of >45% and would have been detected in a TS-based screening program. However, prevalence of joint symptoms, diabetes, cardiac arrythmias, fatigue, skin darkening, and impotence was no higher in the homozygotes than in the 21,375 (10,432 M, av age 57.3; 10,943 F, av age 57.3) wt/wt controls or in heterozygotes. The only difference was a slightly increased prevalence of abnormal liver function tests. These results were not altered by stratifying by age, ferritin level, or TS. Most important, there was no significant difference between the age distribution of the homozygotes and controls. Since HH is a late onset disease, this rules out the possibility that we had studied an essentially healthy population, those with severe manifestations of disease having been systematically excluded. There were, however, two patients among the homozygotes, both with considerable alcohol intake, who might meet the clinical criteria of hemochromatosis. HH can be a severe, potentially fatal disease, and it is important for physicians to detect and treat the severe form of the disease. However, the concept that HH is a common disease is a misconception based upon substitution of biochemical criteria for clinical ones. The cost of screening per life saved or health restored is therefore much higher than has been proposed.

Program Nr: 173 from the 2001 ASHG Annual Meeting

Analysis of 100 Cystic Fibrosis Mutations in 92 Patients with Congenital Absence of the Vas Deferens by Mass Spectrometry. *Z. Wang[1], J.M. Milunsky[1], M. Yamin[1], T.A. Maher[1], R. Oates[2], A. Milunsky[1]*. 1) Center for Human Genetics and Department of Pediatrics, Boston University School of Medicine, Boston, MA; 2) Department of Urology, Boston University School of Medicine and Boston Medical Center, Boston, MA.

Routine, but limited, mutation analysis for CBAVD men has revealed only a minority in which 2 distinct mutations were detected. We sought to determine whether a more extensive mutation analysis might prove to be of benefit for risk estimation and possible prenatal diagnosis later. We studied a cohort of 92 men with CBAVD using MALDI-TOF mass spectrometry (MS) to analyze an approximately hierarchical set of the most common 100 CF mutations. We first demonstrated the accuracy of the assay we developed and the utility of MS as a diagnostic tool with its inherent qualities of high accuracy and ease for automation. 33/92 (35.9%) patients were found to have 2 mutations and 28/92 (30.4%) patients had 1 mutation, while 31/92 (34.7%) patients failed to show any detectable mutations. Compound heterozygosity of severe/mild or mild/mild mutations accounts for 94% (31/33) of the patients with 2 mutations; none of the patients had a genotype of severe/severe mutations that was found in CF patients. CF 100 mutation analysis detected 4.3% more homozygotes and compound heterozygotes and 7.6% more heterozygotes when compared with the 25-mutation panel recommended by the American College of Medical Genetics for couples seeking prenatal care or planning a pregnancy. It has also led to identification of a second mutation in over 50% of those considered to be heterozygotes under the 25-mutation panel analysis. To extrapolate the benefits of CF 100 analysis in general population screening, our data was analyzed excluding IVS8-5T. CF 100 mutation analysis revealed 5/13 (38%) additional mutations and would identify 1 newly recognized carrier per every 7 carriers found under the 25-mutation analysis. Hence, the CF 100 panel provides significantly greater opportunities for routine mutation detection, but especially for partners of known CF carriers and the partners of men with CBAVD.

Program Nr: 174 from the 2001 ASHG Annual Meeting

Diagnostic testing for Prader-Willi Syndrome (PWS) and Angelman Syndrome (AS) a cost comparison. *A. Wiktor, K.G. Monaghan, D.L. Van Dyke.* Department of Medical Genetics, Henry Ford Health System, Detroit, MI.

In 1997, we recommended an approach to diagnostic testing of PWS and AS that considered cost as a factor (Am J Hum Genet 1997;60:244-247). Three strategies were evaluated. Approach IA begins with DNA methylation studies; any positive DNA result is confirmed by FISH. Approach IB begins with FISH analysis; any FISH negative result is followed by DNA testing. Approach II, as described by the ASHG/ACMG Test and Technology Transfer Committee, does DNA testing and FISH simultaneously (Am J Hum Genet 1996;58:1085-1088). Between July 1998 and May 2001, 77 samples were submitted to us for both karyotype and DNA methylation analysis for PWS or AS. Of the 77 cases, 65 had normal karyotype and methylation; 1 had normal karyotype and FISH and positive methylation (uniparental disomy, UPD); 1 had der(6;15)t(6;15)mat, normal FISH and positive methylation (UPD); 3 had del(15), abnormal FISH and abnormal methylation (15q deletion); 4 had normal karyotype, abnormal FISH and abnormal methylation (15q deletion); and 3 had an abnormal karyotype but not del(15), and normal methylation. Based on our actual results, we compared the cost of each strategy assuming $200 for FISH and $300 for DNA testing. A karyotype was done in every case, so that cost was not considered. Thus, the costs for Approaches IA, IB and II would have been $24,900, $37,600 and $38,500, respectively. The cost savings would have been $12,700 for Approach IA vs. IB and $13,600 for IA vs. II. We have adopted Approach IA. For hypotonic infants, where reporting time is critical, we perform FISH and DNA testing simultaneously (Approach II). We request a heparinized blood sample for karyotype and FISH testing, and an EDTA sample for DNA testing. If an EDTA specimen is not drawn, the heparinized sample is shared between the cytogenetics and DNA laboratories. This protocol has resulted in the ability to obtain DNA results in 27 of 28 cases (96.4%) submitted with heparinized blood only. Our experience has shown that Approach IA is cost effective and clinically appropriate.

Program Nr: 175 from the 2001 ASHG Annual Meeting

Linking infant death records to tandem mass spectrometry (MS/MS) newborn screen results in North Carolina.
S.E. McCandless, E.B. Davis, D.M. Frazier, J. Muenzer. Dept Peds, Genetics & Metabol, Univ of North Carolina, Chapel Hill, NC.

Background: North Carolina began population based expanded MS/MS newborn screening as a pilot study in conjunction with Neogen Screening, Inc., Pittsburgh, PA in August 1997. The assumption was made that patients whose first MS/MS newborn screen was borderline, but whose repeat screen was normal, did not have identifiable metabolic disease and were at low risk of death or serious complication.

Methods: To test this assumption we reviewed all North Carolina death certificates of children under the age of 1 year from 1998. Each death was assigned to one of two categories: “known” or “unknown” cause of death. Those death certificates with a clearly non-metabolic cause of death were assigned to the known group (e.g., hypoplastic left heart syndrome, chromosomal anomaly, or motor vehicle accident). All others were assigned to the unknown group (e.g., sudden infant death syndrome, cardiac arrest or epilepsy).

Results:1,011 death certificates of children born after the onset of MS/MS newborn screening were reviewed. 530 infants died before 24 hours of age when the newborn screen is typically drawn and were excluded from the study. A pair of premature twins that died from a urea cycle disorder identified by newborn screening were also excluded. Of the remaining 479 infants, 281 were assigned to the known cause of death group and 198 to the unknown cause of death group. Seven infants had one abnormal MS/MS newborn screen, 5 (1.8%) in the known group and 2 (1.0%) in the unknown group. Chi-square analysis revealed no significant difference between the groups.

Conclusions: There was no significant difference in the frequency of borderline abnormal MS/MS newborn screen results between those infants dying in the first year of life of well-defined non-metabolic conditions and those infants with SIDS or other less clear cut causes of death. This preliminary analysis supports the assumption that borderline MS/MS newborn screen results that are normal on repeat are not associated with an increased risk of infant death in the first year of life.

Program Nr: 176 from the 2001 ASHG Annual Meeting

A Regional Military Healthcare System Response to Expanding Genetic Research: Ethical, Legal and Social Implications. *L.S. Martin, K.A. Azarow, F. Olmsted, J. Daniels, M. Fries, B.C. Calhoun, J. Bullock, R.F. Hume.* Madigan Army Medical Ctr, Tacoma, WA.

Advances in biotechnology, molecular genetic diagnostic capabilities, and the potential for gene therapy incited an explosion of innovative efforts within our existing healthcare system. Contemporaneously, the Military Healthcare System embraced a massive re-engineering process adapting principles of managed care in a rapidly right-sizing environment. These forces required an interdisciplinary learning response in which legal, regulatory and medical models were integrated with the new knowledge in genetics, information management systems, and best business practice methods. How do you perform a business case analysis to provide decision-makers with intelligible choices which can be translated into viable business plans for expanding genetic services? This required Institutional Review Board oversight of multiple clinical investigation demonstration projects: familial breast cancer susceptibility, prenatal screening and diagnosis, newborn screening, genetic research on existing tissure archives, and the genetics of occupational health in the Military Healthcare System. Advances were incorporated in the existing legal framework and regulatory procedures for the protection of research subjects from unintended consequences and unrecognized risks. Our collaborative initiative has resulted in the introduction of new policies for genetic research governance, expanded genetic services, and educational outreach. The critical role of genetic counseling is now recognized within our system. Most important has been the recognition that the genetic paradigm, rather than community health or medical models, optimizes the matching of genetic care for our patients while respecting the multicultural diversity (molecular, social, and religious) of the military community. The most recent NIGHR-ELSI conference supported such an approach to expanding genetics within unique communties. In all of these efforts we have enjoyed the full support of the Northwest Lead Agent, MEDCOM CIRO, and Health Affairs.

Program Nr: 177 from the 2001 ASHG Annual Meeting

Polyglutamine-expanded ataxin-7 induces a cone-rod dystrophy in transgenic mice by antagonizing the function of the nuclear transcription factor CRX. *A.R. La Spada[1], Y.H. Fu[2], B.L. Sopher[1], R.T. Libby[1], X. Wang[3], L.Y. Li[3], D.D. Einum[4], J. Huang[5], D.E. Possin[5], J.B. Hurley[6,7], L.J. Ptacek[4,8], S. Chen[3].* 1) Dept Lab Medicine, Univ Washington, Seattle, WA; 2) Dept Neurobiol & Anat, Univ Utah, Salt Lake City, UT; 3) Dept Ophthal & Vis Sciences, Washington Univ, St Louis, MO; 4) Dept Human Genet, Univ Utah, Salt Lake City, UT; 5) Dept Ophthal, Univ Washington, Seattle, WA; 6) Dept Biochem, Univ Washington, Seattle, WA; 7) HHMI, Univ Washington, Seattle, WA; 8) HHMI, Univ Utah, Salt Lake City, UT.

Spinocerebellar ataxia type 7 (SCA7) is caused by the expansion of a CAG repeat. Although all polyglutamine diseases share a common mutational motif, the basis of cell-type specificity in each disorder remains elusive. To determine the mechanism of ataxin-7 neurotoxicity, we produced transgenic mice that express ataxin-7 with 24 or 92 glutamines. Histological analysis of the 92Q mice revealed periodic thinning of the photoreceptor cell layer of the retina. Whole mounts and immunostaining with pigment-specific antibodies indicated that this periodic thinning is due to preferential loss of cones. ERGs performed on the 92Q mice revealed that they were blind. These results indicate that the process of retinal degeneration in our SCA7 transgenic mice is a cone-rod dystrophy phenotype remarkably akin to what occurs in SCA7 patients. When a yeast two-hybrid assay indicated that the cone-rod homeodomain protein (CRX) interacts with ataxin-7, we performed further studies to assess the significance of this interaction. We found that ataxin-7 and CRX co-localize in nuclear aggregates and can be co-immunoprecipitated. We observed that polyglutamine-expanded ataxin-7 can suppress CRX transactivation. Electrophoretic mobility shift assays performed on retinal nuclear extracts revealed a marked reduction in the ability of CRX to bind its consensus sequence. We carried out real-time RT-PCR analysis on presymptomatic mice and found significant decreases in the expression of genes regulated by CRX. Our results suggest that CRX transcription interference accounts for SCA7 retinal degeneration, and thus may provide an explanation for how cell-type specificity is achieved in this disease.

Program Nr: 178 from the 2001 ASHG Annual Meeting

A bias for CTG/CAG repeat expansions in a primate DNA replication system: Evidence for a cis-element. *C.E. Pearson[1,2], K. Nichol[1], Y.-H. Wang[3], J.D. Cleary[1,2].* 1) Dept Genetics & Genomic Biology, The Hospital for Sick Children, Toronto, ON, Canada; 2) University of Toronto; 3) Dept. Biochemistry, Robert Wood Johnson Medical School, Piscataway, NJ, USA.

Expansions of CTG/CAG repeats is the cause of at least 11 human diseases, including myotonic dystrophy, spinalbulbar muscular atrophy and Huntingtons disease. Repeat instability is intimately connected to the length of the repeat tract: Generally (CTG)n tracts from 4 to 25 repeats are stable and tracts from 34-90 repeats are unstable, displaying a bias for expansions. The mechanism of repeat instability is unknown but may involve DNA replication slippage. In addition, cis-elements in the vicinity of the repeat contribute to instability, however the exact nature of these elements remains unknown. One possible cis-element is the location of replication origins relative to the repeat.

We investigated the effect of the location of replication initiation on CTG/CAG stability in a primate DNA replication system. Replication templates were constructed with the SV40 viral replication origin in one of seven locations relative to repeat tracts of non-affected (17 repeats) or premutation (79 repeats) lengths. Templates were transfected and replicated in primate cells (COS1). Following DNA replication, repeat instability was only observed in premutation length templates. Depending on the distance between the SV40 origin of replication and the repeat tract, these templates yielded predominantly expansions or predominantly deletions or remained intact. The relative location of replication initiation was a more critical determinant of repeat instability than was replication direction. All templates with 17 repeats were stable. This is the first system to recapitulate the repeat length effect and the bias for expansions observed in affected families. Furthermore, our results provide the first evidence that may explain the variable levels of CTG/CAG instability observed in different chromosomal contexts.

Program Nr: 179 from the 2001 ASHG Annual Meeting

Polyglutamine Binding Peptide 1 (QBP1) inhibits polyglutamine aggregation and cytotoxicity *in vitro*and in an *in vivo*disease model. *Y. Nagai[1,2,3], H. Ren[2], J.R. Burke[2], W.J. Strittmatter[2], N. Fujikake[3], H. Higashiyama[3], M. Yamaguchi[4], T. Toda[1]*. 1) Div of Functional Genomics, Dept of Post-Genomics and Diseases, Osaka University Graduate School of Medicine, Suita, Japan; 2) Dept of Medicine (Neurology), Duke University Medical Center, Durham, NC; 3) Dept of Molecular Medical Science, Osaka Bioscience Institute, Suita, Japan; 4) Div of Biochemistry, Cell Biology Sec, Aichi Cancer Center Research Institute, Nagoya, Japan.

Proteins with expanded polyglutamine (polyQ) stretches are responsible for at least eight inherited neurodegenerative diseases, including Huntington's disease. Expansion of the polyglutamine (polyQ) stretch is considered to confer toxic properties on the disease proteins through alterations in their conformation, leading to pathogenic protein-protein interactions and/or to aggregate formation. Molecules that selectively bind the expanded polyQ stretch may interfere with such pathogenic properties, and therefore may be useful as a therapeutic tool. We previously identified Polyglutamine Binding Peptide 1 (QBP1), a peptide sequence that preferentially binds the expanded polyQ stretch using phage display libraries expressing combinatorial peptides. Here, we show the ability of QBP1 to inhibit thioredoxin-polyQ protein aggregation *in vitro*in a concentration-dependent manner. QBP1 almost completely inhibited thioredoxin-Q62 aggregation at one third the concentration of the thioredoxin-Q62 protein. Co-expression of QBP1-CFP with polyQ-YFP in COS-7 cells suppressed polyQ aggregate formation and polyQ-induced cell death (aggregate inhibition; Q45, 39%, Q57, 26%, Q81, 11%, cell death inhibition; Q57, 19%). Moreover, in a *Drosophila*polyQ disease model, QBP1 expression partially rescued polyQ-mediated eye degeneration. Selective inhibition of the pathogenic interactions of expanded polyQ stretches by QBP1 may be a useful strategy for developing potential therapies for the polyQ diseases.

Program Nr: 180 from the 2001 ASHG Annual Meeting

Myotonia is associated with loss of transmembrane chloride conductance and aberrant splicing of Clcn1, the skeletal muscle chloride channel, in a transgenic model of myotonic dystrophy (DM1). *A.K. Mankodi[1], M. Takahashi[2], C. Beck[3], S. Cannon[2], C.A. Thornton[1]*. 1) Univ Rochester, Rochester, NY; 2) MGH Hospital, Boston, MA; 3) Jefferson Univ, Philadelphia, PA.

DM1 is caused by expansion of an untranslated CTG repeat in the DMPK gene. Pathogenic effects of this mutation are likely mediated, at least in part, by the expanded CUG repeat in mutant mRNA. DM1 is characterized by myotonia, a state of hyperexcitability in muscle fibers. In other disorders, myotonia results from defects in the muscle chloride or sodium channels. The pathophysiology of myotonia in DM1, however, is unclear. Lines of transgenic mice (HSA-LR mice) that express expanded CUG repeats develop myopathy and myotonia (Mankodi et al, 2000). We investigated the pathogenesis of myotonia in HSA-LR mice. Intracellular recordings show that hyperexcitability is intrinsic to the muscle fibers, independent of their innervation. Transmembrane chloride conductance is reduced 7.5-fold in HSA-LR mice. Immunofluorescence studies show segmental loss of full-length Clcn1 protein from muscle fibers. DM1 is associated with aberrant splicing of cardiac troponin T (Phillips et al, 1998). We examined the splicing of Clcn1 mRNA in HSA-LR skeletal muscle. RT-PCR experiments show inclusion of a novel Clcn1 exon in HSA-LR mice, similar to the pattern of splicing in muscle from neonatal wild-type mice. Inclusion of this exon results in frame-shift and premature termination at codon 340 of 994. Mis-splicing of this exon correlates with the presence of myotonia in different HSA-LR founder lines. Aberrant splicing of Clcn1, however, is not confined to this single exon. 29 Clcn1 cDNA clones generated from HSA-LR muscle show 11 different splice variants, none of which are observed in wild-type muscle. By contrast, 19 of 20 alpha-sarcoglycan cDNA clones from HSA-LR mice are spliced correctly, an indication that the fidelity of splicing is not universally impaired. We conclude that the nuclear accumulation of expanded CUG repeats triggers aberrant splicing of Clcn1 mRNA. The resulting loss of Clcn1 protein and transmembrane chloride conductance is sufficient to account for the myotonia in HSA-LR mice.

Program Nr: 181 from the 2001 ASHG Annual Meeting

Myotonic Dystrophy Type 2 is Caused by a CCTG Expansion in Intron 1 of *ZNF9*. *L.P.W. Ranum[1], C.L. Liquori[1], M.L. Moseley[1], J.F. Jacobsen[1], A.V. Phillips[2], R. Savkur[2], W. Kress[3], S.L. Naylor[4], T.A. Cooper[2], K. Ricker[3], J.W. Day[1]*. 1) Institute of Human Genetics, Univ Minnesota, Minneapolis, MN; 2) Department of Pathology, Baylor College of Medicine, Houston, TX; 3) Department of Neurology, University of Würzburg, Germany; 4) Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX.

Myotonic dystrophy (DM) can be caused by a mutation on chromosome 19 (DM1) or 3 (DM2/PROMM). DM1 is caused by a CTG expansion in the 3' UTR of the *dystrophia myotonica-protein kinase* gene (*DMPK*). Several mechanisms have been suggested to explain how this mutation causes the multisystemic effects of DM including: DMPK haploinsufficiency; reduced expression of regional genes (eg *SIX5*); and pathogenic effects of the CUG expansion in RNA. Mouse models have suggested that each of these mechanisms contributes to DM1 pathogenesis and that DM1 is a regional gene disorder. To clarify the pathogenic mechanism of DM, we have identified a second human mutation that causes the same multisystemic effects. Linkage disequilibrium analysis (see Liquori, *et al*.) refined the DM2 region. One of our markers, *CL3N58*, showed an aberrant segregation pattern by PCR. Southern analysis demonstrated that all affected individuals in six DM2 families (LOD=31.6 at Q=0.00) had an expanded allele that was not found in controls (n=1360). Sequence analysis showed that the CCTG portion of the compound repeat (TG)n(TCTG)n(CCTG)n expands in affected alleles. The range of expanded alleles is broad (~75-11,000 CCTGs, mean~5,000). The expansion is located in intron 1 of the *zinc finger protein 9* (*ZNF9*) gene, whose normal function as an RNA binding protein appears unrelated to any of the genes found in the DM1 region. Similar to the CUG RNA foci in DM1, intense CCUG-containing nuclear foci were found in DM2 but not control muscle. RT-PCR of insulin receptor (IR) mRNA in skeletal muscle showed a marked reduction in the IR-B isoform in DM2 (18%, n=3) and DM1 (21%, n=9) compared to controls (73%, n=5). Parallels between DM1 and DM2 indicate that the CUG and CCUG expansions in RNA can themselves be pathogenic and cause the multisystemic features common to both diseases.

Program Nr: 182 from the 2001 ASHG Annual Meeting

SELECTION AND ENCEPHALITOGENIC POTENTIAL OF T-CELLS SPECIFIC FOR MYELIN BASIC PROTEIN PEPTIDE [AMINO ACIDS 111-129] USING TRANSGENIC MOUSE WITH HLA-DRB1*0401-IE AND CHIMERIC HUMAN T CELL RECEPTOR. *M.N. Baig[1,2], J. Shukaliak[2], K. Yao[2], S. Ludwin[3], P. Muraro1[2], R. Martin[2], H. McFarland[2], K. Ito[2].* 1) Dept of Genetics, Howard Univ., Wash. DC; 2) Neuroimmunology Branch,NINDS,NIH, Bethesda, MD; 3) Queens University, Ontario, Canada.

Multiple sclerosis (MS) is an inflammatory demyelinating disease that affects the myelin sheath of the central nervous system (CNS). Although the etiology of MS is unknown, myelin-specific CD4+ T-cells are considered essential for disease pathogenesis. Previous characterization of cellular immunity in MS-associated HLA-DR4 (B1*0401) indicates a predominant response toward myelin basic protein peptide of amino acids 111-129 (MBP111-129) in MS patients and normal individuals. In order to study how these MBP111-129-specific T cells are able to escape central tolerance and their role in disease pathogenesis we generated a double transgenic mice expressing human-mouse chimeric HLA-DRB1*0401-IE MHC class II molecule and human-mouse chimeric T cell receptor (TCR). The transgenic TCR (Tg+) composed of human V(D)J region of MS-patient-derived CD4+ T-cell clone and mouse C region with specificity for MBP111-129 and HLA-DRB1*0401 restriction. The Tg+ T cells were positively selected by HLA-DRB1*0401-IE in the thymus, skewed to CD4+ T cell lineage and showed appropriate specificity and restriction. Tg+ T cells activated in vitro induced EAE in irradiated HLA-DRB1*0401-IE transgenic mice and not naive HLA-DRB1*0401-IE transgenic mice. Presences of endogenous T cells appear to reduced disease susceptibility and severity. Disease course was clinically heterogeneous,similar to MS patients. Histological analysis showed preferential localization of inflammatory lesions in the spinal cord and brain stem. When Tg+ mice were crossed with Rag-1-/- mice to eliminate endogenous TCR rearrangement, there was a significant reduction in peripheral T cell frequency. Hybridoma generation and transcriptional analysis suggest that escape of thymic negative selection of the Tg+ T cells is mediated through co-expression of endogenous TCR V-alpha chains. Thus, dual endogenous TCR expression appears essential for selection of autoreactive Tg+ T cells.

Program Nr: 183 from the 2001 ASHG Annual Meeting

Vestibular dysfunction in the Epistatic circler mouse is caused by phenotypic interaction of at least 4 different genes. *G. Van Camp[1], M.P. Van Spaendonck[2,3], K. Flothmann[1], A.M. van Alphen[4], P.H. Van de Heyning[3], J.P. Timmermans[2], C.I. De Zeeuw[4], K. Cryns[1]*. 1) Dept Medical Genetics, Univ Antwerp - UIA, Antwerp, Belgium; 2) Cell Biology and Histology, Univ Antwerp - RUCA, Antwerp, Belgium; 3) Otolaryngology, Univ Antwerp - UIA, Antwerp, Belgium; 4) Anatomy, Erasmus University, Rotterdam, Netherlands.

The Epistatic circler mouse (Ecl-mouse) was used to study the complex pathogenesis of imbalance. This mutant exists in a proportion of the F2-generation from the cross between C57L/J and SWR/J mouse strains and shows circling behaviour indicative for vestibular dysfunction. The present study deals with the genetic, morphological and functional basis responsible for the circling behaviour in the Ecl-mouse. Genetic investigation indicated that the circling behaviour is caused by a single recessively inherited gene derived from SWR/J (the Ecs-gene) in combination with at least 3 different non-recessive genes derived from C57L/J (the Ecl-genes). Genetic mapping made it possible to localise the Ecs-gene to chromosome 14 and the Ecl-genes to chromosome 3, 4 and 13. Vestibular dysfunction in the Ecl-mouse is the first discontinuous genetic defect proven to be caused simultaneously by at least 4 different genes. Morphological examination of the inner ears revealed a bilateral malformation of the horizontal (lateral) semicircular canal. Investigation of the vestibulo-ocular reflex (VOR) in Ecl-mice showed that their horizontal VOR on stimulation is virtually absent, which correlates with the morphological findings. The present data show for the first time that the horizontal and vertical vestibular system of a mouse mutant can be differently and specifically affected. Further research on he Ecl-mouse and identification of the Ecl- and Ecs-genes will lead to a better understanding of semicircular canal development.

Program Nr: 184 from the 2001 ASHG Annual Meeting

Functional analysis of *ABCR*(*ABCA4*) mutations associated with Stargardt disease (STGD), retinitis pigmentosa (RP) and age-related macular degeneration (AMD). *N.F. Shroyer, R.A. Lewis, A.N. Yatsenko, T.G. Wensel, J.R. Lupski.* Baylor College of Medicine, Houston, TX.

Mutations in *ABCR* have been reported to cause a spectrum of recessively inherited retinopathies including STGD and RP, and are associated with the multifactorial disease AMD. We hypothesized that mutant *ABCR* alleles could be asociated with more than one of these diseases in a single pedigree. We screened for *ABCR* mutations in one family that manifests both STGD and RP, and 22 families with STGD and AMD. We found missense and complex alleles associated with STGD, RP and AMD. We demonstrate that AMD-affected relatives of STGD probands are significantly (P = 0.038) more likely to be carriers of *ABCR* mutations than predicted by random assortment of these alleles within the families. Functional analysis of eight AMD-associated mutant alleles by western blotting and azido-ATP labeling of mutant proteins shows measurable defects in 7/8 AMD-associated mutations. Biochemical analysis of both RP-associated mutant alleles (Val767Asp and the complex allele [Trp1408Arg; Arg1640Trp]) show a severe reduction in protein expression and ATP-binding, and therefore these mutations are likely to be functionally null. Interestingly, analysis of the [Trp1408Arg; Arg1640Trp] allele suggests a synergistic effect of the constituent mutations, as both have near wild-type levels of protein and ATP-binding activity. These data reveal that (1) missense mutations in *ABCR* can be associated with both RP and AMD, as well as STGD; and (2) that carrier relatives of STGD patients are predisposed to develop AMD. These data are congruent with our model in which the severity of retinal disease is inversely related to residual *ABCR* activity: RP is associated with homozygous null mutations, and individuals heterozygous for *ABCR* mutations are predisposed to develop AMD.

Program Nr: 185 from the 2001 ASHG Annual Meeting

Autosomal Dominant Mutations Affect X Inactivation Choice. *I. Percec[1,2], R.M. Plenge[2], J.H. Nadeau[2], H.F. Willard[2], M.S. Bartolomei[1]*. 1) HHMI, Univ Pennsylvania, Philadelphia, PA; 2) Case Western Reserve Univ, Cleveland, OH.

X inactivation is the mechanism used by mammals to equalize X-linked gene expression between males and females. It results in the silencing of most genes on one X chromosome during female embryogenesis. While the basic features of this process have been well established, the genetic and molecular mechanisms remain poorly understood. Control of inactivation requires elements within the X inactivation center (*Xic*) on the X chromosome, including the *Xist* gene and a regulatory locus, *Xce*, that distorts the X inactivation ratio in *Xce* heterozygous female mice. While all factors known to be necessary for silencing map to this region, it has long been posited that unidentified autosomal factors are essential to the process. To identify such novel factors, we have developed a phenotype-driven *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis screen for dominant mutations that disrupt X inactivation patterns in female mice. We have assayed 346 G_1 females and have identified dominant mutations that perturb X inactivation patterns in two independent pedigrees. Carrier *Xce* heterozygous females exhibit a shift of their X inactivation ratio from ~30:70 to ~50:50, suggesting an interaction between these mutations and the *Xce* locus. We have demonstrated the dominant transmission of the mutant phenotypes for at least six generations in both pedigrees. The effect appears to be specific to X inactivation, as genomic imprinting of the *H19* gene is not perturbed in either pedigree. Furthermore, the mutant phenotypes are present in all tissues examined, suggesting the mutations act early in the pathway. Importantly, both pedigrees display autosomal modes of inheritance. A genome scan of one pedigree demonstrates linkage of the mutant phenotype to a ~30 cM region of chromosome 15. Genotyping of the other pedigree has revealed that the mutations are not allelic variants. Significantly, these results represent the first evidence of an autosomal mutation affecting any component of the X inactivation pathway. The identification of factors targeted by these mutations and their interaction with *Xce* and other components of the *Xic* will be crucial to understanding the pathway of X inactivation.

Program Nr: 186 from the 2001 ASHG Annual Meeting

Mutations in the perlecan gene of Schwartz-Jampel syndrome: critical role of perlecan in neuromuscular junction functions. *E. Arikawa-Hirasawa[1], A.H. Le[1], I. Nishino[2], I. Nonaka[2], N.C. Ho[3,4], C.A. Francomano[3], P. Govindra[5], J.R. Hassell[5], J.M. Devaney[6], J. Spranger[7], R.E. Stevenson[7], S. Iannaccone[8], M.C. Dalakas[9], Y. Yamada[1].*
1) NIDCR, NIH, Bethesda, MD; 2) National Institute of Neuroscience, Kodaira, Japan; 3) NIA, NIH, Baltimore, MD; 4) Johns Hopkins Bayview Medical Center, Johns Hopkins University Baltimore, MD; 5) Shriners Hospital for Children, Tampa, FL; 6) Research Center for Genetic Medicine, Washington, DC; 7) Greenwood Genetic Center, Greenwood, SC; 8) Texas Scottish Rite Hospital, Dallas, TX; 9) NINDS, NIH, Bethesda, MD.

Perlecan, a heparan sulfate proteoglycan, is present in all basement membranes and in other extracellular matrices and is implicated in cell growth and differentiation. Mutations in the perlecan gene (*HSPG2*) were shown to cause two types of skeletal disorders; severe neonatal lethal dyssegmental dysplasia, Silverman-Handmaker type (DDSH) and the milder Schwartz-Jampel syndrome (SJS). We previously showed that DDSH was caused by functional null mutations of HSPG. SJS is a rare autosomal recessive disorder characterized by chondrodystrophic myotonia. In this report, we have identified five different mutations in *HSPG2* of three unrelated SJS patients. Heterozygous mutations, including splicing mutations and an exon fusion mutation, in two SJS patients produced either truncated perlecan that lacked domain V or significantly reduced levels of wild type perlecan. The third patient had a homozygous 7-kb deletion near the end of *HSPG2* extending to the 3 flanking region that resulted in a reduced amount of nearly full-length perlecan lacking ~35 C-terminal amino acids. Unlike DDSH, the SJS mutations result in different forms and levels of perlecan that are secreted to the extracellular matrix of tissues in SJS patients. We also found reduced amounts of perlecan and acetylcholinesterase (AChE) at the neuromuscular junction (NMJ) in the SJS patients. Reduced clustering of AChE at the NMJ likely causes the myotonia observed in SJS patients. Our results indicate the important role of perlecan in neuromuscular functions and cartilage formation.

Program Nr: 187 from the 2001 ASHG Annual Meeting

Evidence for X chromosome controlling element (XCE) alleles in humans using a population-based association study. *J.M. Amos-Landgraf, H.F. Willard.* Case Western Reserve University and University Hospitals of Cleveland. Cleveland, OH.

The determination of which X chromosome in female mammals is chosen to be inactivated is believed to be a random event whereby either X has an equal probability of becoming the inactive X. However, alleles have been identified in inbred mouse strains that control X chromosome inactivation (XCI) patterns; alleles at the X controlling element (Xce) locus map to the X inactivation center and influence XCI ratios in heterozygous mice. To determine if similar XCE alleles exist in humans, we identified genetic markers in and surrounding the XIST locus and constructed haplotypes to relate these polymorphisms to observed XCI patterns. We sequenced 30 kb of the XIST gene in 15 unrelated X chromosomes, identifying 17 single nucleotide changes and 5 insertion/deletion changes. Half of the variants were found only on two independent X chromosomes, identifying a distinct haplotype that extends more than 50 kb. Phylogenetic analysis of primate XIST genes revealed that the minor alleles at these variant sites represent the ancestral haplotype.

As baseline data, XCI ratios were first determined in blood DNA samples from 915 females (584 newborns and 331 women of childbearing age). XCI ratios from both groups of samples showed a normal distribution pattern, with the means of their distributions centering around 50:50. However, the variance of the two distributions differed significantly ($p=0.0008$), indicating an increase in skewing in females from the time of birth to adulthood. To search for potential human XCE variants, 9 XIST polymorphisms, with minor allele frequencies of between 5% -50%, were genotyped in >450 newborn females. Analysis of two of the XIST polymorphisms revealed genotype-specific variation in the distribution of XCI patterns, with heterozygotes exhibiting a greater degree of skewing ($p< 0.05$), consistent with predictions of the mouse Xce model. The two polymorphisms thus identify haplotypes that define distinct alleles that can influence the observed patterns of X inactivation in heterozygous females.

Program Nr: 188 from the 2001 ASHG Annual Meeting

A Gene for Human Testis Determination Maps to Chromosome 5. *H. Ostrer[1], D. Jawaheer[2], S-H. Juo[3], C. Petit[4], A. Damle[2], S. Dowbak[2], P. Gregersen[2], K. MeElreavey[4].* 1) New York University School of Medicine, New York, NY; 2) North Shore University Hospital, Manhasset, NY; 3) Columbia University College of Physicians and Surgeons, New York, NY; 4) Institut Pasteur, Paris, France.

The induction of testicular differentiation in the embryonic gonadal ridge in mammals is genetically controlled. Most genes in the testis-determining pathway were cloned from the DNA of individuals with constitutional cytogenetic alterations and 46,XY gonadal dysgenesis. The existence of other genes in the pathway has been inferred from familial cases of sex reversal, including 46,XX maleness or true hermaphroditism, or 46,XY mixed or pure gonadal dysgenesis. We report a genome-wide linkage analysis on an extended families recruited from France. Among 76 descendants in four generations there were seven women with 46,XY pure or mixed gonadal dysgenesis and four men with hypospadias or other genital anomalies. Unlike most familial cases of sex reversal, the phenotypic features appeared to be confined to the genitourinary system. DNA was obtained from peripheral blood for 58 members of the family, including all of the affected. A total of 355 microsatellite markers spaced at ~10 cM intervals on 22 autosomal chromosomes from the Marshfield Clinic Set8A were typed. A novel gene for testis determination was mapped to the pericentric region of chromosome 5. A maximal two-point LOD score of 5.03 at theta equals zero was observed at D5S633, assuming sex-limited, autosomal dominant inheritance with a penetrance of 0.99. Linkage was confirmed at several adjacent markers in this region. This study represents the first time that a gene for testis determination in humans was mapped by linkage analysis. Because few mammalian models of genetic sex reversal are known, families such as this represent one of the best available means for identifying new genes for human testis determination.

Program Nr: 189 from the 2001 ASHG Annual Meeting

Genetic dissection of Hirschsprung disease (HSCR). *A. McCallion, E. Stames, A. Chakravarti and 13.* Institute of Genetic Medicine, JHU, Baltimore, MD.

Discrepencies between human and mouse models of HSCR have raised questions concerning the utility of the mouse system in the genetic dissection of this complex disease. Mouse models of HSCR harbor mutations at the same loci as human HSCR patients but they are are observed in the context of a homogenous genome. These are mainly complete loss of function mutations which result in aganglionosis only in homozygotes but do so with full penetrance. This is in stark contrast with human HSCR patients. The vast majority of isolated HSCR cases are compatible with an oligogenic model of transmission, and a number of studies from our laboratory have now proposed a combined role of mutations at RET and EDNRB in HSCR patients. We have intercrossed mice of genotype $Ret^{-}/+$ and $Ednrb^{s}/Ednrb^{s}$, to asses the potential for interaction between these loci (+, -, and s correspond to wild-type, targeted deletion and the hypomorphic mutation piebald, respectively). Mice of these genotypes do not demonstrate an enteric phenotype. We fail to observe an enteric phenotype amongst the >30 compound heterozygotes ($Ret^{-}/+$; $Ednrb^{s}/+$). However, we have also analyzed 74 backcross offspring ($Ret^{-}/+$; $Ednrb^{s}/+$ x Ret+/+; $Ednrb^{s}/Ednrb^{s}$) and make the following observations. Firstly, mice of genotype $Ret^{-}/+$; $Ednrb^{s}/Ednrb^{s}$ are severely affected, and comprise 19%; (expected 25%) of the offspring generated. The majority of these animals demonstrate abdominal distension and/or distress by three weeks post partum. The majority of males observed (8 of 9) demonstrate aganglionosis between the rectum and the splenic flexure and are clearly distressed by 3 weeks post partum. The five affected females examined to date demonstrate variation in the extent of aganglionosis and the age at presentation (death/distress 24 hours after birth to 6 weeks post partum). These experiments clearly demonstrate that pathways compromised in HSCR may be subject to mutation dosage effects, and that these two pathways interact. Observed phenotype and sex differences are likely to result from the influence of mutations at other loci. Our data clearly indicate that the variation in HSCR phenotype expression and sex bias observed in man can be recapitulated in the mouse.

Program Nr: 190 from the 2001 ASHG Annual Meeting

A Genome-wide Scan Identifies a Genetic Locus for Migraine with Aura. *M. Wessman[1, 3], M. Kallela[4], G. Oswell[1], M. Kaunisto[3], J. Hartiala[2], P. Broas[2], E. Hämäläinen[2], P. Marttila[2], T. Hiekkalinna[2], G. Joslyn[2], J. Papp[2], S.M. Leal[5], R. Cantor[2], E. Sobel[2], J. Ott[5], H. Havanka[6], M. Färkkilä[4], L. Peltonen[2], A. Palotie[1]*. 1) Dept of Pathology, University of California, Los Angeles, USA; 2) Dept of Human Genetics, University of California, Los Angeles, USA; 3) Dept of Biosciences, University of Helsinki, Helsinki, Finland; 4) Dept of Neurology, University of Helsinki, Helsinki, Finland; 5) Lab of Statistical Genetics, Rockefeller University, New York, USA; 6) Dept of Neurology, Länsi-Pohja Central Hospital, Kemi, Finland.

Migraine is a common neurovascular disorder with two main types: without aura, occurring in 85% of patients; and with aura (MA), occurring in 15% of patients. MA attacks are preceded or accompanied by transient focal neurological, usually visual, symptoms. Family, twin, and population-based studies support a strong genetic component for MA. Here we report the first genome-wide scan that shows statistically significant linkage for non-hemiplegic migraine. Clinically well-defined MA families from the genetically isolated Finnish population were collected. The ancestors of the families were genealogically traced in population records. A team of clinicians, using a specifically designed questionnaire and life-long information in health care registers, collected details of the disease phenotype. We selected 484 individuals from 55 families demonstrating specific aggregation of MA phenotypes. The scan was performed with 356 markers. SimWalk2 was used to detect genotyping errors. Using MLINK, preliminary two-point analyses revealed linkage of MA phenotype to a chromosome 4 marker yielding a lod score of 3.75. Three adjacent markers provided lod scores above 1.50. PseudoMarker, a model-free likelihood-based analysis using pseudomarker genotypes, gives a lod score of 3.96. Our genome scan thus provides evidence for the first genetic locus in common migraine. Lod scores greater than 1.0 for seven other loci on six chromosomes were observed and are currently targets for further analyses.

Program Nr: 191 from the 2001 ASHG Annual Meeting

Identification of a DNA variant associated with adult type hypolactasia. *I.E. Jarvela[1,2,3], N. Sabri Enattah[2], T. Sahi[4], E. Savilahti[5], J.D. Terwilliger[6], L. Peltonen[2,3,7].* 1) Lab Molecular Genetics, Helsinki Univ Hosp, Helsinki, Finland; 2) Dept Molecular Medicine, National Public Health Institute, Biomedicum, Haartmanink 8, Helsinki,Finland; 3) Dept Medical Genetics, Univ Helsinki, Finland; 4) Dept of Public Health, Univ Helsinki, Finland; 5) Hosp Children and Adolesc, Univ Helsinki, Finland; 6) Dept Psychiatry and Columbia Genome Center, NY, USA; 7) Dept Hum Genet, UCLA,Los Angeles,CA, USA.

Adult-type hypolactasia (lactose intolerance) is an autosomal recessive condition resulting from physiological decline of the lactase-phlorizin hydrolase (LPH) enzyme activity in intestinal cells. Using linkage, allelic association and extended haplotype analysis carried out in nine extended Finnish families we restricted the adult-type hypolactasia locus to a 47 kb interval at the distance from the LPH gene on 2q21. The sequence analysis of the region revealed a single nucleotide polymorphism (SNP), C/T that completely cosegregates with adult-type hypolactasia in all Finnish families and in a sample set of 236 individuals from four different populations. Another SNP (G/A) residing 8 kb telomeric from the C/T-variant was associated with the trait in all but 7 cases. SNP-analysis in 1047 DNA samples reflected the reported prevalence of adult-type hypolactasia in three different populations. Based on the linkage disequilibrium on persistence alleles, the introduction of the persistence variant to the Finnish population has occurred 9000-11400 years ago, in good agreement with initiation of the dairy culture. Identification of the same DNA variant in hypolactasia alleles from several populations should stimulate studies of the evolutionary origin of this interesting variant and provides basis for the development of a diagnostic DNA-test for adult-type hypolactasia.

Program Nr: 192 from the 2001 ASHG Annual Meeting

An examination of gene-environment interaction in a linkage study of asthma and household smoking. *S. Colilla[1], C. Ober[1], T. Beaty[2], E. Bleecker[3], M. Blumenthal[4], S. Rich[3], N. Cox[1] and the CSGA.* 1) Human Genetics, University of Chicago, Chicago, IL; 2) Johns Hopkins University, Baltimore, MD; 3) Wake Forest University, Winston-Salem, NC; 4) University of Minnesota, Minneapolis, MN.

Asthma is a common, chronic disease of the airways, which is considered to have a multifactorial etiology involving both genetic and environmental factors. Linkage mapping of genes for asthma has been challenging. We tested the hypothesis that inclusion of household smoking exposure would affect the ability to detect linkage for asthma. Using 144 multigenerational Caucasian families from the Collaborative Study for the Genetics of Asthma (CSGA), we contrasted results of a baseline multipoint linkage analysis (Allegro, Gudbjartsson et al., 2000), using 323 autosomal markers across 22 chromosomes, with results obtained using 0/1 weights to represent household smoking exposure status of a family. Families were classified as exposed if they had at least one asthmatic who was exposed to household smoking as a baby. Results examining if household smoking exposure as an infant would increase LOD score, showed a significant increase in LOD score for a region on 2q (D2S1776, 173 cM, $p = 0.05$). Regions where the LOD score became nominally significant after weighting for household smoking included 1p (D1S551, 110 cM), 4q (D4S1625, 133 cM), 5q (D5S816, 135 cM), and 10q (D10S1225, 76 cM). To investigate these regions further, we did a linkage analysis considering only asthmatic pairs who were both exposed to smoking in their household while a baby. The results from chromosome 2 for D2S1776 showed a similar significant increase in LOD score (all asthmatic pairs' LOD = 0.07; only exposed asthmatic pairs' LOD = 1.14, $p < 0.05$). A significant increase in LOD score was also observed for 5q at D5S816 when analyzing only exposed asthmatic pairs (all asthmatic pairs' LOD = 0.34; only exposed asthmatic pairs' LOD = 1.23, $p < 0.05$). Overall, these results suggest that incorporating environmental exposure information into a linkage analysis may increase the ability to detect linkage in a complex trait such as asthma, particularly if a gene-environment interaction is suspected to be involved.

Program Nr: 193 from the 2001 ASHG Annual Meeting

Fine linkage and disequilibrium mapping of human obesity loci in chromosome region 7q22.3-7q35. *W. Li, R.A. Price.* Dept Psychiatry, Behavioral Genetics, Univ Pennsylvania Medical Ctr, Philadelphia, PA.

Although leptin is an important hormone in body weight regulation and linkage results suggests the region of chromosome 7 containing the leptin gene co-segregates with extreme obesity, leptin coding region mutations are rare. To investigate whether leptin flanking sequence and/or a larger 40 cM region (7q22.3-7q35) contribute to extreme obesity, we genotyped 249 families segregating extreme obesity (203 Eurpoean-American, 44 African-Americans, 2 others, 1277 subjects) using 20 microsatellite markers and two single nucleotide polymorphisms (SNPs). We also carried out transmission disequilibrium analyses (TDT) for 159 triads (135 Caucasians, 24 African Americans) using 19 markers (including 4 SNPs). Both quantitative (SOLAR and MAPMAKER/SIBS) and qualitative (GENEHUNTER) analyses provided evidence of linkage for BMI, percent fat, waist circumference, and waist/hip ratio, with D7S692 (npl=3.02, 20cM centromeric to leptin). The leptin region and three other regions (D7S1804-D7S2452, D7S685 and D7S2202-D7S794) gave suggestive evidence for linkage. In African American triads, we found a common four-SNP haplotype just 5' of leptin (within 2.5 kb) associated with obesity (chi square=11.27, p=0.00079). However, the same tendency was not found in European American triads, although gave suggestive evidence for linkage further 5'. We found linkage disequilibrium for D7S692(transmitted/untransmitted=153/101, chi-square=10.65, p=0.0011), D7S2501(370 kb 5' to leptin, transmitted/untransmitted=106/61, chi2=12.13, p=0.000497) and D7S2438(7.4 cM 3' to leptin, transmitted/untranmitted=44/18, chi2=10.9, p=0.00096). We also found a four-markers haplotype (D7S2459-D7S692-D7S523-D7S643) associated with BMI (transmitted/untransmitted=7/0,chi2=7,p=0.0082). Our results suggest two or more obesity related genes lie within thergion 7q22-35. (Supported in part by NIH grants R01DK44073, R01DK58095 and R01DK56210 to RAP).

Program Nr: 194 from the 2001 ASHG Annual Meeting

Genome-wide screen for essential hypertension in a deep rooted pedigree from an isolated Sardinian subpopulation. *A. Angius[1], GB. Maestrale[1], P. Forabosco[1], E. Petretto[1], G. Casu[1], D. Piras[1], M. Fanciulli[1], PM. Melis[1], M. Palermo[2], M. Pirastu[1]*. 1) Istituto Genetica Molecolare,CNR,Alghero; 2) Istituto Endocrinologia,Università Sassari, Italy.

The Sardinian village of Talana exhibits features such as slow demographic growth, high endogamy and inbreeding, reduced genetic heterogeneity, low number of founders, stable culture, accurate genealogical and demographic records ideal for the study of complex disorders. Clinical assessment of the entire adult population (1000) identified about 150 hypertensive subjects (DBP >90 mm Hg). We selected the most severe cases (DBP >100 mm Hg) belonging to a single deep-rooted pedigree (11 generations), whose common ancestors lived in the 17th century. Our strategy is based on the use of all the genealogical information in order to minimize the number of cases required. In addition, analysis of a single pedigree decreases the probability of heterogeneity and increases the chance of finding major and minor effect genes. We performed a 2-stage genome-wide scan. In stage 1, we typed 15 affected individuals and their close relatives with 400 markers (at 10 cM intervals). We carried out affected-only linkage and allele sharing analyses with FASTLINK and SIMWALK2 programs. Allele frequencies in the population were estimated from 25 healthy unrelated individuals. Statistics with suggestive results (lod score >1.2; p-value <0.05) were found on chromosomes 2, 13, 15 and 17. We carried out stage 2 on these four loci with larger number of affected subjects belonging to the same extended pedigree using markers spaced at ~2 cM intervals. We found higher lod scores for all these regions. In particular a lod score of 3.26 and 3.13 was detected on D15S205 under affected-only recessive and dominant model respectively. The localization on chromosome 2 (lod=1.86 for D2S287) was strongly supported by non-parametric statistics (p-value <0.005) indicating an excess of IBD-sharing among all affected. Further investigation of these regions with increased number of markers will allow fine mapping and the possible identification of candidate genes in these loci.

Program Nr: 195 from the 2001 ASHG Annual Meeting

SNP haplotypes in the angiotensin I-converting enzyme (ACE): analysis of Nigerian family data using gamete competition models. *C.A. McKenzie[1], A. Adeyemo[2], J.S. Sinsheimer[3], N. Bouzekri[4], L. Southam[5], A. Hugill[5], X. Zhu[6], T.E. Forrester[1], R. Cooper[6], R.D. Cox[5], G.M. Lathrop[5]*. 1) Tropical Metabolism Research Unit, University of the West Indies, Kingston, Jamaica; 2) Dept. of Pediatrics/Institute for Child Health, University of Ibadan, Ibadan, Nigeria; 3) Dept. of Human Genetics, Biomathematics and Biostatistics, UCLA, Los Angeles, CA; 4) Institute of Biological Anthropology, University of Oxford, Oxford, UK; 5) Mammalian Genetics Unit, Medical Research Council, Harwell, Oxford, UK; 6) Dept. of Preventive Medicine and Epidemiology, Loyola University Medical Center, Maywood, IL.

Circulating ACE levels are influenced by a quantitative trait locus (QTL) which is either within or near to the ACE gene. In British Caucasian families the major QTL lies downstream of an ancestral breakpoint located near to position 6435 in the gene. In addition, several markers in the 3' end of the gene are in very strong linkage disequilibrium (LD) with this QTL. These results are supported by analyses in Jamaican, Nigerian and French Caucasian families. It has been reported that there is also evidence of a 2nd QTL in strong LD with markers in the 5' end of the ACE gene in Nigerian and French Caucasian families. Gamete competition models have recently been introduced as a generalization of the transmission/disequilibrium test. These models have been implemented in the software package MENDEL and allow the use of full pedigree data and quantitative as well as qualitative traits. Gamete competition models may also be extended to the analysis of haplotypes formed by intragenic markers. As part of the International Collaborative Study of Hypertension in Blacks (ICSHIB), 13 biallelic markers in the ACE gene were typed in 1,343 Nigerians from 332 families. We have applied gamete competition models to these data. Several markers in both 5' and 3' ends of the gene are associated with ACE concentration ($P< 10^{-6}$). We also explore the relationship between marker haplotypes and ACE concentration, and use well-established model selection techniques in order to quantify the independent effects of markers found on different haplotypes.

Program Nr: 196 from the 2001 ASHG Annual Meeting

Quantitative trait loci (QTL) genome scan in two independent sets of Finnish affected sibling pair families with type 2 diabetes. *L.J. Scott[1], R.M. Watanabe[2], K. Silander[3], K.L. Mohlke[3], H.M. Stringham[1], C. Li[1], K. Doheny[4], E. Pugh[4], T.T. Valle[5], R.N. Bergman[6], J. Tuomilehto[5], F. Collins[2], M. Boehnke[1]*. 1) Dept Biostat, Univ Michigan, Ann Arbor, MI; 2) Dept Prevent Med, Keck Sch Med, Univ S California, Los Angeles, CA; 3) NHGRI, NIH, Bethesda, MD; 4) CIDR, Johns Hopkins Univ, Baltimore, MD; 5) Dept Epidemiol Hlth Promotion, Nat Pub Hlth Inst, Helsinki, Finland; 6) Dept Physiol Biophys, Keck Sch Med, Univ S California, Los Angeles, CA.

The goal of the Finland-U.S. Investigation of NIDDM Genetics (FUSION) is to identify genes that predispose to type 2 diabetes and/or influence diabetes related quantitative traits. In a previous genome scan of 580 families (F1), we examined QTL for insulin, glucose, and obesity related traits using variance components on affected individuals. We subsequently collected a second set of 269 families (F2), on which 477 microsatellite markers were typed. The F1 and F2 probands had similar characteristics but the F2 group tended to be milder diabetics. The highest maximum lod score (MLS) in the combined F1+F2 groups was 4.1 for C-peptide (CP) based empirical insulin secretion (IRC=CP/glucose) at 56.5 cM from the p-ter on chromosome 3 (Chr 3). Specifically, for F1, F2 and F1+F2, IRC had MLSs of 3.6, 1.2, and 4.1 (64.0, 56.5, 56.5 cM) and C-peptide had MLSs of 2.7, 1.5 and 3.5 (63.5, 56.5, 56.5 cM). On Chr 7, for F1, F2 and F1+F2, fasting glucose had MLSs of 1.7, 1.1 and 2.7 (80.0, 66.0, 79.0 cM) and IRC had MLSs of 2.3, 1.5 and 2.4 (76.5, 54.0, 71.0 cM). In addition to traits included in the original F1 QTL genome scan, we examined lipid and blood pressure variables in both F1 and F2. On Chr 7, triglyceride (TG), HDL ratio (HDL/total cholesterol) and cholesterol had MLSs in F1 of 1.4, 3.5, and 2.3 (135.5, 142.0, 142.5 cM), respectively (no signals in F2). On Chr 20 the MLSs for TG were 1.4, 2.0, and 1.9 (70.0, 83.5, 82.5 cM) in F1, F2, and F1+F2, respectively. This TG peak overlaps with an F1 Chr 20 linkage peak (MLS = 2.3, 69.0 cM), although no or minimal linkage peaks (lod <0.5) were found in the QTL regions on Chr 3 and 7.

Program Nr: 197 from the 2001 ASHG Annual Meeting

Human centromeres are organized into distinct linear and spatial domains. *B. Sullivan, G. Karpen.* Molecular and Cell Biology Lab, Salk Institute, San Diego, CA.

Centromeres in fission yeast and Drosophila contain multiple domains that contribute to kinetochore assembly, sister chromatid cohesion and heterochromatin formation. We have studied the organization of human centromeres using indirect immunofluorescence with antibodies to centromere proteins (CENPs). At metaphase, CENP antibodies typically are seen as double dots by conventional two-dimensional (2-D) fluorescence microscopy. In three dimensions (3-D), CENP antibodies appear as cylindrical tubes extending along the width of the outer face of the chromatids. This cylindrical higher-order structure is also observed at Drosophila centromeres. The CENP-A cylinder is located closest to the chromatin, CENP-C overlaps minimally with CENP-A, and CENP-E does not overlap with CENP-A at all. We further analyzed centromeric organization at even higher resolution by mapping DNA and proteins onto extended chromatin fibers. The kinetochore, as defined by CENP-A binding, is present on only half of the alpha satellite array. The remaining portion of alpha satellite DNA is being mapped relative to the cohesion protein hRAD21 and heterochromatin proteins HP1 and SUVAR3-9. In contrast to previous studies in humans, but concurring with studies of Drosophila centromeres, human kinetochores contain both H3 and CENP-A nucleosomes in a repeated subunit structure. We estimate that 1/3 to 1/2 of the region contains CENP-A nucleosomes, indicating that only a subset of the centromeric chromatin actually contributes to kinetochore structure and function. CENP-C also localizes along the same length of chromatin as CENP-A in extended fibers. The 2-D results from chromatin fibers suggest that eukaryotic centromeric chromatin from yeasts to humans consists of multiple structural and functional domains. We propose that the linear repeats of H3 and CENP-A nucleosomes within kinetochore chromatin give rise to a 3-D helical or cylindrical structure that exposes CENP-A but not H3 to outer kinetochore and spindle proteins.

Program Nr: 198 from the 2001 ASHG Annual Meeting

Functional centromere DNA is structurally encoded. *J.E. Koch.* Cancercytogenetics Laboratory, Department of Hematology, Aarhus Amtssygehus, Aarhus, Denmark.

Alpha satellite DNA forms the DNA component of natural human centromeres. It is composed of 171bp monomers which are organized into higher order repeats. The higher order repeat organization seems of functional importance as the alpha satellite has one matrix attachment (MAR) site per higher order repeat. A monomer contains a variable and a conserved region, and the conserved region comprises two regions of dyad symmetry (symmetries 1 & 2) and an intervening $(GT)_2$ element.

The functional importance of these data has been elusive. However, we have now found that symmetry 1 *in vitro* forms a hairpin structure which is recognized and cleaved by the enzyme topoisomerase II (Topo II), whereas the corresponding B-form DNA is not cleaved. Topo II concentrates at centromeres in mitosis and its activity is needed for successful chromosome segregation. We have further found that Topo II *in vivo* cleaves alpha satellite DNA at active centromeres, but not at inactive centromeres. The activity at active centromeres is restricted to a domain within the alpha satellite. Thus, the activity is not directed against the alpha satellite *per se*, but rather against a particular form of that DNA. Combining *in vivo* and *in vitro* data we propose that the active centromere DNA is found in a hairpin conformation, wheras the inactive centromere DNA is found in standard B-form, and that this hairpin form carries the DNA contribution to the centromere function. This interpretation is supported by the existence of similar symmetry elements in centromere DNA from other sources, including DNA from a human neocentromere, and by our observation of the centromeric Topo II activity also at a human neocentromere.

From our findings and previous knowledge we have derived a model that explains how the hairpin DNA is formed and what it's functional significance is. According to this model, the hairpin conformation is a result of DNA replication and the very recognizable appearence of the hairpin DNA is important both for defining the localization of the active centromere and for the execution of centromere function.

Program Nr: 199 from the 2001 ASHG Annual Meeting

Artificial chromosomes carrying X alpha satellite as an assay for centromere function. *M.K. Rudd, H.F. Willard.* Case Western Reserve University and University Hospitals of Cleveland. Cleveland, OH.

Despite the importance of centromeres to ensure proper segregation of chromosomes during mitosis and meiosis, the minimal sequence requirements for human centromere function have not been identified. We are defining the minimal human centromere using the X chromosome centromere as a model. Like all human centromeres, the X centromere is composed of alpha satellite DNA. X alpha satellite exists as a chromosome-specific higher order repeat (DXZ1) extending over several Mb adjacent to a less extensive, but divergent array of heterogeneous monomers that do not demonstrate higher-order structure (see abstract by Schueler). While the genomic organization of X alpha satellite is known, the precise region of alpha satellite responsible for conferring centromere function is unknown. To address which DNA sequences comprise the X chromosome centromere, we tested candidate alpha satellite sequences for their ability to generate de novo centromeres in an artificial chromosome assay. When 85 kb of DXZ1 was transfected into human HT1080 cells together with a selectable marker, artificial chromosomes containing DXZ1 were generated in ~ 10% of clones. Four such artificial chromosomes were selected for further study and characterization. The amount of DXZ1 DNA contained in the artificial chromosomes ranged from an estimated <500 kb to over 5 Mb. Each was mitotically stable in the absence of selection and recruited centromere proteins (CENP-E) associated with active centromeres, suggesting that the artificial chromosomes have assembled functional centromeres with DXZ1 DNA. While DXZ1 is the third higher-order repeat unit to be shown capable of forming de novo centromeres, the human alpha satellite DNA family is unusually heterogeneous in both structure and sequence. Thus, the artificial chromosome system should allow testing of a range of candidate DNA sequences capable of forming de novo centromeres, including various classes of monomeric X alpha satellite that map adjacent to DXZ1. These data complement data from Xp and Xq isochromosomes that address whether other regions of alpha satellite adjacent to DXZ1 are capable of centromere function.

Program Nr: 200 from the 2001 ASHG Annual Meeting

Identification of two distinct genomic organizations in neocentromeres: implications for neocentromere acquisition. *D.L. Satinover, E.E. Eichler, S. Schwartz.* Dept Genetics, Case Western Reserve Univ. and Univ. Hosp, Cleveland, OH.

Eight years have passed since the original report of a neocentric chromosome. More than 43 neocentromeres later, little insight has been gained on the understanding of neocentromere activation. Our lab has focused on characterizing neocentromeres using cytogenetic and molecular methods to define the sequences involved in neocentromeric activity. Previous work in our lab has defined a common neocentromeric region on 9p23 cytogenetically to ~500 kb and further refined by chromatin immunoprecipitation (ChIP) experiments to ~380 kb. Extensive sequence analysis has been performed with this region and compared to two other neocentromeric regions (10q25 and 20p12) defined by ChIP. Not surprisingly, no satellite sequences have been found in this region on 9 and examination of tandem repeats revealed random distribution throughout the region. However, our neocentromeric region is highly AT-rich, equal to levels of alpha satellite DNA. We have found a significant decrease in Short Interspersed Nuclear Elements (4.29% SINEs) and a significant increase in Long Terminal Repeat sequences (20.87% LTR) when compared to the average of randomly obtained genomic segments of equal size.

Sequence analysis of our neocentromere and comparison to others reveal several important findings: (1) The neocentromeric region from 9p23 has a significant increase in LTRs and a significant decrease in SINEs; (2) No obvious pattern is shared among the three extensively studied neocentromeres, eliminating the possibility of a magic sequence; and most importantly (3) We have shown that neocentromeres from 9p23 and 10q25 share similar properties including enrichment of several AT-rich 7mer sequences, elevated levels of retransposable elements, and increases in the level of low complexity DNA compared to the randomly obtained genomic segments of similar size and opposite to the neocentromere from 20p12. Our findings suggest that primary sequences may not be as important as generalized sequence composition in providing the proper environment required to gain the epigenetic mark for neocentromere activation.

Program Nr: 201 from the 2001 ASHG Annual Meeting

Molecular and cytogenetic studies of spreading of X inactivation in four X;autosome translocations. *A.J. Sharp, D.O. Robinson, P.A. Jacobs.* Wessex Regional Genetics Lab., Salisbury District Hospital, Salisbury, Wiltshire, England.

We have used allele-specific RT-PCR and late-replication studies to determine the spread of X inactivation through autosomal chromatin in four unbalanced X;autosome translocations. In the first case, 46,X,der(X)t(X;10)(q26.3;q23.3), 4 genes spread over 30Mb of autosomal DNA were inactive on the der(X;10), while the most distal gene examined remained active. In contrast to this spread of inactivation and the patients normal phenotype, no spreading of late-replication into the translocated 10q was observed. Similar long-range, but incomplete, inactivation was also observed in the second case, 46,X,der(X)t(X;11)(q26.1;p11.2). Inactivation within 11p11-p15.4 was apparently continuous, as demonstrated by the silencing of 7 genes, accompanied by CpG methylation. However, inactivation within 11p15.5 was discontinuous, with the 5 most distal genes tested showing varying activity. Consistent with transcription and phenotype data, there was a spreading of late-replication through most of the translocated 11p. Two further cases, both of which involve similar regions of 6p, were also studied. In both cases long-range inactivation was observed, but the pattern of gene silencing was both discontinuous and incomplete. Pairwise observations of individual genes revealed both concordant and discordant inactivation between the two cases, suggesting that the spread of X inactivation through autosomal DNA is not dictated solely by sequence-specific factors. Observations of late-replication were also discordant between the two der(X;6) chromosomes. No spreading of late-replication was observed in one case, while a variable but discontinuous pattern was seen in the second, with a late-replicating region confined to distal 6p in a proportion of cells. Cytological observations of H4 acetylation and XIST RNA also showed a similar distribution in this latter case. We conclude that long-range silencing of autosomal genes by X inactivation can occur without many of the features normally associated with the inactive X. *asharp@hgmp.mrc.ac.uk.*

Program Nr: 202 from the 2001 ASHG Annual Meeting

A new immunological approach for examination of human meiotic exchange. *A. Lynn[1], L. Judis[1], E. Chan[1], A. Seftel[1,2], T. Hassold[1,2].* 1) Department of Genetics, Case Western Reserve University, Cleveland, OH; 2) University Hospitals of Cleveland, Cleveland, OH.

Until recently, studies of meiotic exchange in humans have been based upon chiasma counts from diakinesis preparations or on linkage studies of progeny. Each has limitations: the former relies on preparations that are difficult, at best, to analyze while the latter involves indirect inference from recombination patterns observed in post-selecion zygotes. However, we can now overcome these limitations: recent reports suggest that MLH1, a *MutL* homolog, localizes to sites of meiotic crossing over. We have initiated studies of human exchanges, using immunolocalization methodology to examine the distribution of MLH1 foci on synaptonemal complexes, and FISH to identify a subset of chromosomes (1, 16, 21 and 22). To date, we have analyzed pachytene cells from testicular biopsies of 11 individuals with obstructive azoospermia but normal spermatogenesis on histology; on average 73 cells/individual were analyzed. Our results are consistent with those expected for a molecule that "marks" the sites of exchange. An average of 50 MLH1 foci/cell was observed which, as expected, is equal to approximately twice the average number of recombinations observed from CEPH genotype data (~27 recombinations in male meioses). Further, the position of exchanges on chromosomes 1, 16, 21 and 22 (i.e., centromeric, medial, distal) were similar to patterns observed from CEPH families. Evidence for crossover interference was observed both in the number of exchange events per chromosome and in their relative spacing. We observed considerable intra- and inter-individual variation in total number of MLH1 foci; in most individuals, the range was 40-60 MLH1 foci/cell, while mean values among individuals ranged from 47 to 54 foci. There was no affect of age on rate of meiotic exchange. Unexpected rare findings included chromosomes with zero exchanges and acrocentric chromosomes with exchanges observed on their p-arms. Our results indicate that this will be a powerful approach for characterizing genome-wide variation in human recombination rates, as well as for analyzing protein-DNA interactions in recombination.

Program Nr: 203 from the 2001 ASHG Annual Meeting

Unexpectedly high rate of de novo constitutional t(11;22)s in sperm from normal males.. *H. Kurahashi[1], B.S. Emanuel[1,2]*. 1) Division of Human Genetics and Molecular Biology, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Pediatrics, University of Pennsylvania School of Medicine, 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. We have generated a PCR-based translocation detection system for the t(11;22) using PCR primers flanking the PATRRs on both chromosomes. To examine the prevalence of de novo occurrence of this translocation, PCR was performed on sperm DNA samples obtained from four normal individuals. All of the sperm samples examined contained the translocated chromosomes detected by PCR in some, but not all aliquots of DNA. The frequency was estimated to be 0.89-7.20x10 $^{-5}$, which is higher than was expected based on the incidence of clinically detected de novo translocation carriers. Lymphoblast and fibroblast DNAs from normal individuals were also analyzed, and none were positive for this translocation. Neither lymphoblasts from Bloom syndrome patients, which show frequent sister chromatid exchange, nor those from patients with ataxia-telangiectasia, another chromosomal breakage syndrome, demonstrated the translocation. These data indicate a meiotic origin for this translocation. Palindromic sequences have been demonstrated to induce double strand breaks during meiosis in yeast. Our data suggest that this translocation, which occurs exclusively in gametes, is generated as the product of a physiological double strand break and repair system during meiotic recombination.

Program Nr: 204 from the 2001 ASHG Annual Meeting

The Recurrent t(11;22): FISH Analysis of 11q23 and 22q11 in Oogenesis. *B.S. Emanuel[1], D. Conforto[1], M.M. Cohen[2]*. 1) Children's Hosp. of Phila; 2) Greater Balt. Med. Ctr.

The t(11;22)(q23;q11) is the only recurrent, non-Robertsonian constitutional translocation. Asymptomatic carriers are often identified after the birth of unbalanced offspring with +der(22)t(11;22) syndrome. In 42 unrelated families, we showed that the 11q23 and 22q11 breakpoints (BPs) are in palindromic AT rich repeats (PATRRs). The remarkable similarity in independent BPs is unprecedented and suggests permissive interactions between these two chromatin domains in interphase nuclei during meiosis. We propose that the t(11;22) occurs after repair of double strand breaks mediated by the PATRRs at the BPs. Further, perhaps proximity, juxtaposition or non-homologous pairing of these sequences in the meiotic interphase nucleus promotes the recurrence of the t(11;22). To address this hypothesis, we have examined the position of the 11q and 22q BP regions, relative to one another in interphase nuclei of meiotic preparations. We performed two color FISH on nuclei of normal fetal oocytes with simultaneous use of four probes. The relative distance between the chromosome 11 and 22 BP region in 50 oocytes was determined by cohybridization with whole chromosome paint (WCP) and unique BP probes for chromosomes 11 and 22. Visualization of intact bivalents was accomplished using one WCP labeled with FITC and the other with rhodamine. The BP regions were identified by single copy probes labeled with the alternative color fluor to the WCP. Control experiments examined the distance between chromosomes 6q and 22q in 50 oocytes, using a unique probe from 6q26 and the 6WCP. Based on visual assessment, only oocytes in zygotene or pachytene were included in the study. Measurements of the distance between 11q23 and 22q11 or 6q26 and 22q11 were computer generated from digitally captured images. The measurements were sorted independently with regard to increasing distance and plotted individually. An obvious tendency for 11q23 and 22q11 to be closer together than 6q26 and 22q11 was observed. Coupled with evidence of an unexpectedly high rate of *de novo* t(11;22)s in sperm from normal males, these studies begin to dissect meiotic events responsible for this recurrent abnormality.

Program Nr: 205 from the 2001 ASHG Annual Meeting

Histone methylation and chromosome stability. *T. Jenuwein.* Institute for Molecular Pathology, The Vienna Biocenter, Vienna.

Heterochromatin has been implicated in the functional organization of chromosomes by defining a specialized chromatin structure at the centromeres. The recent discovery of the first histone lysine methyltransferases (Suv39h HMTases) has allowed the identification of a molecular mechanism in which the specific methylation of histone H3 at lysine 9 (H3-K9) generates a binding site for heterochromatin-associated proteins. Indeed, genetic and biochemical approaches indicate that the murine Suv39h HMTases regulate H3-K9 methylation at pericentric heterochromatin, and that this modification is essential for chromosome stability during mitosis and meiosis. Combined disruption of the two Suv39h HMTases in the mouse germ line results in severely impaired viability and complete spermatogenic failure. Pericentric H3-K9 methylation in somatic and early meiotic cells is lost in the absence of the Suv39h HMTases. Suv39h double null (dn) primary mouse embryonic fibroblasts display increased chromosomal instabilities - a phenotype that is further reflected by the development of B-cell lymphomas in Suv39h mutant mice. Second, in early meiotic prophase of Suv39h dn spermatocytes, chromosomes engage in non-homologous interactions through their centromeric regions and are delayed in synapsis. A significant fraction of meiosis I cells contains mis-segregated chromosome bivalents, and the highly heterochromatic Y chromosome fails to pair with the X chromosome. Together, these data establish a crucial role for H3-K9 methylation in regulating a 'heterochromatic competence' that protects chromosome function and genome stability during mitosis and meiosis.

.

Program Nr: 206 from the 2001 ASHG Annual Meeting

Deficient *in vivo* DNA end-joining in Fanconi anemia fibroblasts. *S.L. Donahue, R. Lundberg, C. Campbell.*
Department of Pharmacology, University of Minnesota Medical School, Minneapolis, MN.

Fanconi anemia (FA) is a rare, inherited cancer predisposition syndrome. Cells from these patients display elevated spontaneous chromosome breaks and hypersensitivity to DNA damaging agents. These findings have led many to speculate that FA is a DNA repair syndrome. Consistent with this view, nuclear extracts from diploid fibroblasts derived from FA patients of complementation groups A, C, and D (FAA, FAC, and FAD fibroblasts, respectively) are defective in an *in vitro* DNA end-joining activity that is distinct from the well-characterized Ku-dependent nonhomologous DNA end-joining pathway. However, rejoining of these same substrates in intact FA lymphoblasts occurs with a frequency that is indistinguishable from that seen in wild-type lymphoblasts. To examine this apparent paradox, we measured plasmid end-joining in intact FA fibroblasts. Linear plasmid DNA molecules were electroporated into wild-type, FAA, and FAD fibroblasts. Following a 48 hour incubation period, plasmid DNA was recovered, and the efficiency with which rejoining occurred determined using a bacterial reporter system. Whereas 40 percent of cohesive-ended plasmid molecules introduced into wild-type fibroblasts were rejoined, FAA and FAD fibroblasts rejoined 7 and 10 percent respectively, of these molecules. Similar analysis of blunt-ended plasmid molecules revealed that wild-type fibroblasts rejoined 27 percent, while FAA and FAD fibroblasts each rejoined 5 percent of these substrates. These findings indicate that FA fibroblasts are deficient in DNA end-joining. The absence of a similar defect in either intact FA lymphoblasts, or nuclear extracts derived from these cells, implies that there are fundamental differences in the way that fibroblasts and lymphoblasts repair DNA double-strand breaks.

Program Nr: 207 from the 2001 ASHG Annual Meeting

WHIM Syndrome, a combined immunodeficiency disease, is caused by mutations in the HIV co-receptor gene *CXCR4*. *P.A. Hernandez[1], R.G. Gorlin[2], J.N. Lukens[3], G.A. Diaz[1]*. 1) Departments of Human Genetics and Pediatrics, Mount Sinai School of Medicine, New York, NY 10029; 2) Department of Oral Biology and Genetics, University of Minnesota School of Dentistry, Minneapolis, MN 55455; 3) Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN 37232.

WHIM syndrome is an immunodeficiency condition in which patients manifest warts, hypogammaglobulinemia, immunodeficiency, and myelokathexis (neutropenia without maturation arrest). All cases have been sporadic or consistent with dominant inheritance, although apparently recessive familial myelokathexis without warts has been reported. Utilizing three pedigrees with a total of 11 affected individuals, a whole-genome scan was performed and the WHIM syndrome disease gene was localized to a ~12-cM region of chromosome 2q21 (maximal multipoint LOD score = 3.01). The critical region spanned ~16.7 Mb and contained 23 known or predicted genes. The morphology of bone marrow neutrophils, as well as recent molecular studies, suggested the occurrence of premature apoptosis. The most attractive candidate with clear relevance to apoptosis or cell cycle regulation was the chemokine receptor *CXCR4*. This gene has been shown to play a critical role in hematopoietic, vascular and brain development in homozygous null mice and in the control of apoptosis in human chronic lymphocytic leukemia B cells. Mutation analysis of affected individuals revealed the presence of two nonsense mutations, 1000C—>T and 1027G—>T, that truncate 19 and 10 residues, respectively, from the C-terminal cytoplasmic tail domain. Patient-derived lymphoblastoid cells carrying the 19-residue truncation showed increased calcium release relative to control lymphoblastoid cells when stimulated with the CXCR4 ligand, SDF-1, consistent with a gain-of-function mechanism. Mutations were not found in recessive cases of myelokathexis, documenting genetic heterogeneity. In summary, we report the assignment of the WHIM syndrome disease gene to chromosome 2q21 and the identification of mutations in the chemokine receptor, *CXCR4*, causing this immunodeficiency condition. These findings constitute the first demonstration of a human disease caused by mutation of a chemokine receptor.

Program Nr: 208 from the 2001 ASHG Annual Meeting

Mutations in DNA ligase IV define a new radiation sensitivity disorder. *K. Cerosaletti[1,2], M. O'Driscoll[3], P.M. Girard[3], S. Palmer[4], R. Gatti[5], P. Jeggo[3], P. Concannon[1,2].* 1) Virginia Mason Research Ctr, Seattle, WA; 2) Univ Washington, Seattle, WA; 3) Univ Sussex, East Sussex,UK; 4) Univ Texas Health Sciences Ctr, San Antonio, TX; 5) UCLA, Los Angeles, CA.

Chromosomal instability and specific sensitivity to ionizing radiation are associated with the autosomal recessive disorders ataxia telangiectasia and Nijmegen Breakage Syndrome (NBS). Patients with NBS are characterized by microcephaly, unusual facial features, growth retardation, immunodeficiency, chromosomal instability, radiation sensitivity, and increased incidence of lymphoid cancers. Most NBS patients have mutations in the NBS1 gene, located on human chromosome 8, which is involved in DNA double-strand break repair. In the course of diagnostic screening for NBS, we have encountered patients with NBS-like symptoms who do not have mutations in the NBS1 gene. We previously reported two siblings with clinical features typical of NBS, as well as myelodysplasia, chronic skin conditions, and hypothyroidism. No mutations were found in the NBS1 gene in the siblings and cell lines from the patients expressed normal levels of nibrin protein. Expression of the Mre11 and Rad50 proteins, which complex with nibrin, was also normal. By contrast, DNA ligase IV enzymatic activity and protein expression was severely reduced in patient cell lines. Sequence analysis of the DNA ligase IV gene revealed two nonsense mutations, 1738C/T > R/X580 and 2440C/T > R/X814. These mutations delete one or both of the C-terminal BRCT domains of the ligase IV protein, which are required for interaction with XRCC4 and protein stabilization. Consistent with reduced ligase IV activity, patient fibroblasts had increased numbers of unresolved DNA breaks. To confirm DNA ligase IV in the etiology of the disorder, a wildtype ligase IV cDNA was introduced into patient fibroblasts. Normal levels of DNA ligase IV protein expression were obtained and ligase IV enzymatic activity was increased, resulting in complementation of the radiation sensitivity of patient fibroblasts. Additional patients with mutations in DNA ligase IV have been identified, defining a new inherited disorder with chromosomal instability and radiosensitivity.

Program Nr: 209 from the 2001 ASHG Annual Meeting

Sepiapterin reductase deficiency: a novel tetrahydrobiopterin-dependent monoamine neurotransmitter deficiency without hyperphenylalaninemia. *N. Blau[1], L. Bonafe[2], J.M. Penzien[3], B. Czarnecki[4], J. Friedman[5], M. Maccollin[5], B. Thony[1]*. 1) Division of Clinical Chemistry and Biochemistry, University Children's Hospital, 8032 Zurich, Switzerland; 2) Division of Metabolism and Molecular Pediatrics, University Childrens Hospital Zrich, Switzerland; 3) Childrens Hospital, Augsburg, Germany; 4) Childrens Hospital Knigsborn, Unna, Germany; 5) Neurogenetics Department, Massachustes General Hospital, Charlestown, MA, USA.

Classical tetrahydrobiopterin (BH4) deficiencies are characterized by hyperphenylalaninemia and deficiency of monoamine neurotransmitters. Here we report three patients with progressive psychomotor retardation, dystonia, severe dopamine and serotonin deficiency (low 5-hydroxyindoleacetic and homovanillic), and abnormal pterin pattern in cerebrospinal fluid (high biopterin and dihydrobiopterin). Furthermore, they all presented with normal urinary pterins and without hyperphenylalaninemia. Investigation of skin fibroblasts revealed inactive sepiapterin reductase (SR), the enzyme catalyzing the final two-step reaction in the biosynthesis of BH4. Mutations in the SPR gene were detected in all three patients and their family members. Two patient were homozygous for a TC>CT dinucleotide exchange, predicting a truncated SR protein Q119X. The third patient was a compound heterozygote for a genomic 5 bp deletion (1397-1401delAGAAC) resulting in abolished SR gene expression, and a A>G transition leading to a R150G amino acid substitution and inactive SR as confirmed by recombinant expression. The absence of hyperphenylalaninemia, normal urinary pterin metabolites, and normal SR-like activity in red blood cells might be explained by alternative pathways for the final two-step reaction of BH4 biosynthesis in peripheral and neuronal tissues. We propose that in peripheral tissues, SR activity may be substituted by the aldose, carbonyl, and dihydrofolate reductases for the biosynthesis of BH4, whereas in the brain only aldose and carbonyl reductase are fully present. Thus, autosomal recessive SR deficiency leads to BH4 and neurotransmitter deficiency without hyperphenylalaninemia and may not be detected by the neonatal screening for phenylketonuria (PKU).

Program Nr: 210 from the 2001 ASHG Annual Meeting

Neonatal intrahepatic cholestasis caused by citrin deficiency: Clinical features of 14 patients. *T. Ohura[1], Y. Tazawa[2], K. Kobayashi[3], D. Abukawa[1], I. Nagata[2], R. Sumazaki[4], Y. Kohno[2], O. Sakamoto[1], K. Iinuma[1], T. Saheki[3].* 1) Dept Pediatrics, Tohoku Univ Sch Med, Sendai, Japan; 2) Dept Pediatrics, Tottori Univ; 3) Dept Biochemistry, Kagoshima Univ; 4) Dept Pediatrics, Tsukuba Univ.

Adult-onset type II citrullinemia (CTLN2) is caused by a deficiency of a novel gene SLC25A13 (Nat Genet 22:159, 1999). The SLC25A13 gene encodes a calcium-binding mitochondrial carrier protein, named citrin. The late onset of serious and recurring symptoms is a hallmark of CTLN2. However, we have detected SLC25A13 mutations in patients with neonatal intrahepatic cholestasis, and the clinical features of neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) have been uncovered (Hum Genet 108:87, 2001). In this study, we investigated 14 NICCD patients to further clarify the clinical presentation of the disease.

Seventeen cholestatic infants with diffuse fatty liver of unknown cause were examined by gene analysis for eight SLC25A13 mutations identified in CTLN2 patients. Fourteen of the patients were proven to be homozygotes (n=5) or compound heterozygotes (n=9) for SLC25A13 mutations. Homozygotes with IVS11+1G>A or compound heterozygotes with 851del4/IVS11+1G>A were frequent (8/14). NICCD patients were referred to our hospitals for three major reasons; i.e.: cholestatic jaundice, vitamin K deficiency, and failure to thrive. Laboratory findings on the 14 patients were as follows: None of patients had hyperammonemia. Serum direct bilirubin ranged from 1.4 to 6.8 mg/dl. Total bile acids were over 120 mmol/L in all patients. g-GTP activities over 100 IU/L were found in 11 of 13 patients examined. Ten patients had low activities of vitamin K-dependent coagulant factors. High blood levels of galactose were found in 4 patients, 2 of whom had cataracts. Analysis of plasma amino acids showed that not only citrulline, but also threonine, methionine, tyrosine, arginine levels were elevated in 9 patients. All patients responded to nutritional management, including fat-soluble vitamins, MCT containing formula and/or lactose-free formula. Our study showed that patients with NICCD present with a variety of clinical signs and biochemical findings.

Program Nr: 211 from the 2001 ASHG Annual Meeting

Mutations in a novel gene, *SUTAL*, cause a unique form of Hermansky-Pudlak syndrome, HPS-3. *M. Huizing[1], Y. Anikster[1], J. White[2], Y. Shevchenko[3], D. Fitzpatrick[1], J. Touchman[3], J. Compton[4], S. Bale[4], R. Swank[5], J. Toro[4,6], W. Gahl[1]*. 1) NICHD, NIH; 2) Univ of Minnesota, MN; 3) ISC, NIH; 4) NIAMS, NIH; 5) Roswell Park, Buffalo, NY; 6) NCI, NIH.

Hermansky-Pudlak syndrome (HPS) is a rare autosomal recessive disorder characterized by oculocutaneous albinism and a storage pool deficiency. Two genes causing HPS have been identified. *HPS1* (causing HPS-1 disease) codes for a protein of unknown function, and *ADTB3A* (causing HPS-2 disease) codes for a subunit of AP-3, a coat protein involved in vesicle formation. However, many HPS patients do not have mutations in either of these genes, so we attempted to identify other HPS-causing genes. Using homozygosity mapping on pooled DNA of 6 families with mild HPS from central Puerto Rico (PR), we localized a new HPS susceptibility gene to chromosome 3q24, corresponding to the locus of the mouse HPS model *subtle gray*. Using database searches, BAC, EST, and cDNA sequence comparisons, 5'-RACE, northern blotting, and cDNA and genomic sequencing, we identified a novel gene, *SUTAL*, with 17 exons and a 3024-bp coding region. A homozygous 3904-bp deletion was present in all central PR patients. The deletion probably arose by homologous recombination across two flanking *Alu* repeats. It includes exon 1 and part of intron 1, and results in absence of *SUTAL* mRNA. Based upon ancestral haplotype analysis, we estimate that the mutation arose approximately 5.3 generations ago, coincident with a local migration within central PR between 1880 and 1890. Besides the central PR mutation, we also identified a homozygous splice site mutation in 3 Ashkenazi Jewish patients, and 6 different mutations in other non-PR patients. The *SUTAL* peptide, sutalin, is predicted to be 114kDa, and cytoplasmic. Sutalin has no homology to any known proteins, but does contain a potential clathrin binding motif, an ER retention signal, and lysosomal targeting signals. Cell biology experiments should reveal the role of sutalin in the formation of vesicles of lysosomal lineage, e.g., melanosomes and platelet dense bodies. In addition, clinical characterization of HPS-3 should allow better prognosis of HPS patients based upon the causative gene.

Program Nr: 212 from the 2001 ASHG Annual Meeting

The human homolog of mouse *light ear* (*le*) is a major gene for Hermansky-Pudlak syndrome (HPS). *T. Suzuki[1], E. Novak[2], Q. Zhang[2], W. Li[2], E. Sviderskaya[3], S. Hill[3], D. Bennett[3], R. Swank[2], R. Spritz[1]*. 1) Hum Med Genet Prog, Univ Colorado Hlth Sci Ctr, Denver, CO; 2) Dept Molec Cell Biol, Roswell Park Cancer Inst, Buffalo, NY; 3) Dept Anat Devel Biol, St. George's Hosp Med Sch, London, UK.

HPS is a rare autosomal recessive disorder characterized by tyrosinase-positive oculocutaneous albinism, bleeding tendency, and a ceroid-lipofuscin lysosomal storage disease, with frequent fatal complications. We previously identified the *HPS1* gene, which accounts for virtually all cases of HPS in Puerto Rico, but only about half of HPS in non-Puerto Rican patients. In the mouse, more than 15 loci are associated with mutant phenotypes similar to human HPS, and we previously showed that one of these, *pale ear* (*ep*), is the mouse homolog to human HPS1.

Another mouse mutant, *light ear* (*le*), has a phenotype identical to that of *ep*. Western blot analysis showed that the HPS1 protein is absent in both *ep* and *le*-mutant mice, suggesting that the HPS1 and le proteins may be part of a complex destabilized by the absence of either protein. Together, these findings suggested *le* as a candidate for a second major HPS locus in humans. Accordingly, we mapped and positionally cloned mouse *le*, located on chromosome 5, in a region homologous to human 22q. The *le* gene encodes a novel, ubiquitously-expressed protein, *le*-mutant mice having a homozygous nonsense mutation. We next characterized the human *le* gene homolog, and screened for mutations in 19 HPS patients lacking mutations in the *HPS1* gene. Six had obvious pathological mutations, confirming the human *le* homolog as a major gene for human HPS. Immunofluorescence analysis of melanotic and non-melanotic cells transfected with FLAG-tagged mouse *le* cDNA showed perinuclear localization in a pattern similar to that of the HPS1 protein. Light and electron microscopy of a permanent line of melanocytes, derived from *le*-mutant mice, showed enlarged melanosomes identical to those seen in *ep*-mutant melanocytes. We are currently characterizing the precise location of the le protein, its possible interaction with HPS1 and other proteins, and its role in biogenesis of melanosomes and lysosomes.

Program Nr: 213 from the 2001 ASHG Annual Meeting

Ectodysplasin-A1 is sufficient to rescue both hair growth and sweat glands in Tabby mice. *A.K. Srivastava[1], M. Durmowicz[2], A.J. Hurtung[1], J. Hudson[1], L. Ouzts[1], D. Donovan[2], C. Cui[2], D. Schlessinger[2].* 1) J. C. Self Res. Inst. of Human Genetics, Greenwood Genetic Center, Greenwood, SC; 2) Lab. of Genetics, Nat. Inst. on Aging, Baltimore, MD.

Mutations in ectodysplasin-A (EDA), a member of the tumor necrosis factor family, are associated with a clinical syndrome characterized by loss of hair, sweat glands and teeth, suggesting its vital role in the development of these ectodermal appendages. When the mouse gene, orthologous to EDA, is defective, it produces the *tabby (Ta)* phenotype, which is also deficient in skin appendages. Among several EDA splice isoforms, the two longest isoforms, EDA-A1 (391 amino acids) and EDA-A2 (389 amino acids) activate NF-kB by binding, respectively, to two distinct receptors, EDAR and XEDAR. We have asked whether the different EDA isoforms are all required in cooperation to produce each type of appendage, or whether they are assigned to specify one or another appendage. We produced a line of *tabby* transgenic (Ta/Y+Tg) mice using the mouse ectodysplasin-A1 cDNA under the control of a CMV promoter. Expression of the ectodysplasin-A1 isoform in *tabby* males showed 70% to complete restoration of hair growth, dermal ridges, and sweat glands, as assessed by scoring of hair behind the ears and on the body and tail, by scanning electron microscopy, and by sweat tests. In contrast to the *Ta* mice (which completely lack hair follicles and associated glands), histological sections show that the number of follicles in the Ta/Y+Tg mice is the same as in wild-type; but the development of individual follicles and associated glands varies from indistinguishable from wild-type to smaller and/or only partially formed. These results indicate that the EDA-A1 isoform is a key regulator of hair follicle and sweat gland initiation. Other ectodysplasin-A isoforms may not be absolutely required for skin appendage formation, but consistent with distinctive temporal and spatial expression of the EDA-A2 isoform, are likely required for appropriate timing and completeness of development.

Program Nr: 214 from the 2001 ASHG Annual Meeting

Altered peripheral T-cell control and multiple features of APECED phenotype in *Aire* Knock out mice. *C. Ramsey[1], O. Winqvist[2], L. Puhakka[1], M. Pelto-Huikko[3], L. Peltonen[1,4,5] and APECED.* 1) Dept of Human Genetics, Univ of California, Los Angeles, Los Angeles, CA, USA; 2) Dept of Internal Medicine, Univ of Uppsala, Uppsala, Sweden; 3) Hospital for Children and Adolescence, Helsinki Univ Hospital, Finland; 4) Dept of Human Molecular Genetics, National Public Health Institute, Helsinki, Finland; 5) Dept of Medical Genetics, Univ of Helsinki, Finland.

Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy (APECED) is a monogenic autosomal recessive disease caused by mutations in the *AIRE* gene on 21q. APECED is a model disease for progressive non-specific HLA linked autoimmunity. The patients show defects of self-tolerance and multiple tissue-specific autoantibodies. We produced the knock-out mouse strain to dissect the molecular pathogenesis of APECED by inserting a neo cassette into exon 6 of the *Aire* gene. 73% of Aire deficient mice demonstrated the presence of autoantibodies against at least one of the four organs tested and 40% of the *Aire*-/- mice sera displayed autoreactivity against two or more of the four tested target organs: liver, testis, pancreas and adrenal glands. Furthermore, 50% of the mice displayed lymphocytic infiltration of the liver and 85% of the *Aire*-/- mice were infertile. Thus, the loss of Aire function leads to a phenotype closely resembling the human APECED disease. Quantitative and qualitative characterization of T-cells did not reveal any differences. No major defects were observed in the antigen processing or in the thymic education of T-cells and no differences were observed in spontaneous activation of T-cells in naive mice, but the loss of proliferative response was evident in the lymph nodes of immunized *Aire*-/- mice. Furthermore, distinct differences were observed in the peripheral, but not in the thymic TCR repertoire. Collectively, our immunological data suggest that the autoimmune manifestation in APECED is due to altered peripheral, but not central tolerance. We would like to propose that AIRE protein serves as a proliferative checkpoint control being crucial in the periphery when T-cells encounter foreign antigens, but not in the central maturation of T-cells.

Program Nr: 215 from the 2001 ASHG Annual Meeting

Mice deleted for the DiGeorge/Velocardiofacial syndrome region have shizophrenia-related behaviour and learning and memory impairments. *R. Paylor[1,2], K.L. McIlwain[1], R. McAnich[1], A. Nellis[1], L.A. Yuva-Paylor[1], A. Baldini[1,3], E.A. Lindsay[3]*. 1) Department of Molecular and Human Genetics, Baylor Coll Medicine, Houston, TX; 2) Division of Neuroscience,Baylor Coll Medicine, Houston, TX; 3) Department of Pediatrics (Cardiology), Baylor Coll Medicine, Houston, TX.

*Del22q11*syndrome, which comprises DiGeorge syndrome and velocardiofacial syndrome, is caused by haploinsufficiency of region of 22q11.2. Children diagnosed with *del22q11*syndrome have an increased risk for developing schizophrenia, schizoaffective disorder and bipolar disorder. These psychiatric disorders develop during adolescence and early adulthood, whereas the more commonly recognized *del22q11*-associated behavioural deficits (learning disabilities, deficits of motor development, attention deficit disorder) are evident in early childhood. It is not known which gene(s) in the deleted region modulates these behaviours. We have modeled the human deletion in the mouse (*Df1*), and here we show that heterozygously deleted mice (*Df1*/+)have schizophrenia-related behaviour, learning and memory deficits. The mice were tested in behavioural tests that assess different CNS functions. *Df1*/+mice showed sensorimotor gating deficits, as measured by prepulse inhibition of the startle response. Significantly, similar deficits occur in patients with schizophrenia and schizotypal personality disorder, where they are thought to underlie the thought fragmentation and attention deficits that afflict patients. *Df1*/+ mice also showed impaired contextual fear conditioning, as measured in the Pavlovian conditioned fear test. Their responses suggest difficulty remembering complex environmental cues, but not simple auditory ones. Together, these results suggest that like patients with schizophrenia, *Df1*/+mice have problems processing complex information. *Df1*/+mice behave like wild type animals in other behavioural tests. Thus, *Df1*/+ mice model at least two features of the complex *del22q11*-associated behavioural phenotype. This finding not only opens the way to pharmacological analyses that may lead to improved treatments, but also to the identification of gene/s that modulate these specific behaviours in humans.

Program Nr: 216 from the 2001 ASHG Annual Meeting

Cloning Great - G Protein-Coupled Receptor Affecting Testicular Descent. *A.I. Agoulnik, I.P. Gorlov.* Dept OB/GYN, Baylor Col Medicine, Houston, TX.

In the human population cryptorchidism or undescended testis occurs in 3-4% of males at birth, making this abnormality the most frequent congenital birth defect in newborn boys. Two main consequences of an abnormality in the location of the testis are infertility, caused by degeneration of the spermatogonial cells, and a high risk of malignant tumors in adulthood. Identification of the genes that play a role in the testicular descent is a key to a better understanding of this abnormality. An autosomal mouse transgene insertional mutation crsp (cryptorchidism with white spotting) has been identified in Baylor College of Medicine (Overbeek et al. Genesis, 2001). The crsp mutation causes high intraabdominal cryptorchidism in homozygous males. Transgene insertion occurred upstream of the Brca2 gene and was accompanied by the deletion of approximately 550 kb of genomic DNA. Through a combination of the positional cloning approaches and sequence data analysis we have identified a novel gene (Great) encoding a protein with a distinct homology to an extended family of glycoprotein hormone G protein-coupled receptors. Restricted expression pattern of the Great gene (gubernaculum, testis, and brain) suggested an involvement in testicular descent. We have successfully targeted the mouse gene in ES cells and obtained germ-line transmission of the null-allele in mice. Complementation analysis of the Great knock-out allele with an original crsp mutation produced cryptorchid, white-spotted phenotype. Thus, the Great gene is responsible for the testicular abnormality in crsp mutant mice.

Program Nr: 217 from the 2001 ASHG Annual Meeting

Linkage disequilibrium within and between multiple X and autosomal genomic regions. *M.E. Zwick[1], D.J. Cutler[1], C.T. Yohn[1], K.P. Tobin[1], C. Kashuk[1], N. Shah[2], J. Warrington[2], A. Chakravarti[1]*. 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins School of Medicine; 2) Affymetrix Inc., Santa Clara, CA.

The mapping of genetic variants underlying complex traits may depend upon the patterns of linkage disequilibrium throughout the human genome. To examine this, we employed high-density probe arrays, to characterize SNP variation in 32 autosomal and 8 X-linked genomic regions, each consisting of 50kb of unique sequence spanning a 100kb locus among forty individuals from the NIH Polymorphism Discovery Resource. Repetitive sequences from each genomic region were masked while unique sequences were amplified using long-range PCR. We screened 2.9 MB in each of 40 individuals for a total of 117MB while identifying 6,229 SNPs. We restricted our analysis to high quality sites as identified with the ABACUS algorithm (Quality Score of 30 or greater).

In our analysis, significant LD consists of a pair of segregating sites that are associated by a Fishers Exact Test at $P<0.001$. Because these results depend upon allele frequencies, we analyze pairs of sites with frequencies such that they could have shown statistical significance. We report four main results. First, X-linked genomic regions show a greater proportion of site pairs with significant linkage disequilibrium than autosomal genomic regions (X-linked: 2394 of 5855, or 41%; Autosomal: 29062 of 131365, or 22%). Second, the median distance between site pairs with significant LD is longer on the autosomes (14.4 kb) than on the X (7.4kb). Third, the median distance varies enormously for both X-linked (4.7-46.2kb) and autosomal (3.5-50kb) genomic regions. Fourth, in spite of the extensive LD variation between genomic regions, lower frequency site pairs have significant LD over longer physical distances ($R^2=0.47$). Our results suggest that the density of markers required for genetic mapping will be highly region dependent. Furthermore, even within a single genomic region, different frequency variants will have significant LD over different physical distances. Thus, the history of a variant is as important as the distance in determining linkage disequilibrium.

Program Nr: 218 from the 2001 ASHG Annual Meeting

The Sequence of Human Chromosome 20 and its Comparative Analysis. *P. Deloukas.* The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, CB10 1SA, Cambridge, UK.

Chromosome 20 is a metacentric chromosome representing 2.2-2.4% of the human genome. We here report the completion of sequencing the euchromatic portion of this chromosome as another milestone in our quest to produce a finished reference human sequence. The clone map which was assembled by fingerprinting and STS content analysis, is in 6 contigs of which one spans the entire p arm. The four gaps in the q arm have all been sized by FISH and together account for less than 300 Kb of DNA. We sequenced a set of 628 overlapping clones and generated 59,191,425 bp of sequence. The finished sequence was analysed on a clone by clone basis using a combination of similarity searches against DNA and protein databases, as well as a series of *ab initio* gene predictions. We annotated 717 genes, and 166 pseudogenes. Excluding pseudogenes, chromosome 20 has a gene density of 12.18 genes/Mb which is intermediate to 6.71 (low) and 16.31 (high) reported for chromosome 21 and 22, respectively. Gene density fluctuates greatly along the chromosome with the q arm being more gene rich. Structural characteristics of the annotated genes and the distribution of predicted features such as CpG islands and transcriptions start sites will be presented. Through analysis of sequence overlaps we identified circa 11,000 new SNPs which we are comparing to the 29,478 SNPs reported earlier in the year, to define a unique set. Our findings on the comparative analysis of chromosome 20 to circa 16 million mouse shotgun reads (Mouse Sequencing Consortium) and to a 2X sequence coverage of the genome of the pufferfish *T. nigroviridis* (Genoscope) will also be discussed. Chromosome 20 is associated with diseases such as Creutzfeld-Jakob disease (PRNP) and Alagille syndrome (JAG1) for which the underlying genetic defect is already known. Through linkage studies chromosome 20 has also been associated to common diseases such type 2 diabetes, obesity, and eczema for which the causative variant(s) remain unknown. The reported finished and annotated sequence and its variation will be a valuable tool in understanding the genetic basis of these and other diseases linked to chromosome 20.

Program Nr: 219 from the 2001 ASHG Annual Meeting

Ensembl: A multi-genome computational platform. *E. Birney[1], M. Clamp[2], A. Kraspcyk[1], G. Slater[1], T. Hubbard[2], V. Curwen[1], A. Stabenau[1], E. Stupka[1], L. Huminiecki[1], S. Potter[2].* 1) Genome Annotation, EBI, Hinxton, UK; 2) Sanger Centre, Hinxton UK.

Ensembl (www.ensembl.org) is an established system for automatic annotation and analysis of large eukaryotic genomes. Currently Ensembl has annotated human and mouse sequence and we are in the process of annotating worm, fly, fugu and mosquito in collaboration with their respective communities

Ensembl provides a number of access points to this data including graphic web based front end system, local java based viewers and direct data access. In all cases we aim to integrate best possible gene prediction using all potential evidence with other resources, such as markers, SNPs, SAGE expression and actively curated genome information

In the area of computational comparative genomics, Ensembl aims to provide as many tools and data resources as possible. We were the first group to provide computational matching of mouse whole genome shotgun reads to the human genome and have refined our gene predictions using this information. In addition we have a system to present genomic regions across multiple vertebrates to a researcher

Ensembl is an entirely open system: the data is usable with no restriction and we have an open source code policy, allowing any group to take advantage of our work. We hope that Ensembl provides useful information for genetics researchers and are interested in feedback and ideas for future improvement.

Program Nr: 220 from the 2001 ASHG Annual Meeting

NCBI's genome annotation projects. *D.R. Maglott, R. Agarwala, H-C. Chen, S. Chetvernin, D. Church, O. Ermolaeva, W. Jang, P. Kitts, J. Kans, D. Lipman, J. Ostell, K. Pruitt, S. Resenchuk, G. Schuler, S. Sherry, T. Tatusova, S. Wheelan.* Natl Ctr Biotechnology Info, Natl Library Medicine, NIH, Bethesda, MD.

Over the past year, NCBI has developed a process to assemble public sequence, compute the position of genes and other important features, and provide unrestricted access to both the data and tools to view it. The process starts with public finished and draft sequence from which contaminants have been removed, and computes an assembly based on sequence alignment, guided by curated overlaps. The result is released as reference sequence (RefSeq) accessions of the format NT_000000. These accessions include curated and computed annotation of genes, mRNAs, coding regions, STS markers, variation, FISH-mapped clones and the GenBank accessions from which the sequence was derived. For each mRNA and protein annotated in that assembly sequence, NCBI also produces mRNA and protein RefSeq records, recognized by their accessions in the format XM_000000 and XP_000000, respectively. Many of the annotated features on the these RefSeq records include links to other resources where related information is available. In addition to explicit annotation, potential gene locations are predicted using GenomeScan and by aligning ESTs.

Products of NCBI's annotation project include: (1) sequence data (the RefSeqs mentioned above as well as coding regions and proteins predicted by Genome Scan); (2)pre-computed protein matches to models and alignments of those matches; (3)genome-specific BLAST and (4)MapViewer. MapViewer has many roles: (1)user-controlled displays of maps in several coordinate systems, (2)Genome View of BLAST results, and (3)display of evidence of expression or conserved sequences based on GenomeScan predictions or EST alignments. The EST maps are actually the combination of two reports: histograms of aligments of EST accessions along the chromosome (a rough indicator of expression) and alignments organized by UniGene cluster. The order of genes and ESTs generated by these analyses are also used to generate comparative maps.

Program Nr: 221 from the 2001 ASHG Annual Meeting

Applying computational methods to search genomic sequence to identify candidate disease genes and novel sequence characteristics. *T.A. Braun[1], T. Scheetz[1,3], K. Mykytyn[2], D. Nishimura[2], T. Casavant[3], V.C. Sheffield[2,4]*. 1) Genetics, Univ of Iowa, Iowa City, IA; 2) Pediatrics, Univ Iowa; 3) Electrical/Computer Engineering, Univ of Iowa; 4) Howard Hughes Medical Institute, Univ of Iowa.

Several valuable data resources have been developed as direct and indirect results of the Human Genome Project. We are developing a system to acquire and mine data from specific databases to aid our efforts to identify disease genes. As part of the system, an application has been developed to automatically acquire draft and finished contigs in candidate genomic intervals that have been assembled and mapped by NCBI. This application downloads multiple contigs from GenBank and stores the GenBank records locally. A high speed cluster of Linux workstations is used to analyze sequence and perform distributed sequence processing and alignments to identify UniGene clusters as part of our data mining and analyses. The cluster of workstations is used to mask repetitive elements and regions of low complexity in the genomic sequence. The workstations also enable large scale distributed BLASTing of multi-megabase regions. A modified version of the UniGene dataset is used to identify distinct clusters in draft and finished genomic sequence from large numbers (1000s) of BLAST results. The process from these applications may also be used to perform the automated extraction of UniGene expression data. These applications have been used to mine biological databases for sequence and expression data within multiple genomic intervals to identify potential candidate disease genes associated with Bardet-Biedl Syndrome (BBS). Applying these applications to a 2cM region on chromosome 3 associated with BBS3, 15 Mb of sequence has been acquired and mined. From this interval, 297 distinct clusters have been identified from the redundant BLAST alignments of 3286 clusters. We have confirmed that the majority of the 100 most frequent clusters map to chromosome 3. This system has also been applied to other larger genomic intervals. Based on this experience with processing large amounts of sequence, we are also developing applications to search genomic sequence for novel characteristics that contribute to mutational events.

Program Nr: 222 from the 2001 ASHG Annual Meeting

A high-throughput cDNA sequencing and gene discovery platform using a concatenated-cDNA-sequencing (CCS) strategy. *P.H. Gunaratne[1], A.M. Garcia[1], S. Hale[1], X. Lu[1], Y. Tsang[2], S. Hulyk[1], L. Gay[1], N. Walsham[1], C. Kowis[1], J. Margolin[2], R. Gibbs[1]*. 1) Human Genome Sequencing Center, Baylor Col Medicine, Houston, TX; 2) Department of Pediatric Hematology & Oncology, Texas Children's Hospital, Houston, TX.

The Baylor-HGSC has established a high-throughput cDNA sequencing pipeline and gene discovery platform using a concatenated-cDNA-sequencing (CCS) strategy developed here. The two major efforts currently ongoing include the NIH Mammalian Gene Collection (MGC) project and the Texas Childrens Hospital (TCH) Project. The MGC project is aimed at the identification and systematic cataloging of full-length cDNA sequences (FLcDNA) and complete ORFs associated with every single gene in human and mouse by a consortium of groups including the Baylor-HGSC. The TCH project is focused on the characterization of FLcDNAs from patients affected with pediatric leukemia. Using this strategy cDNA inserts are purified and concatenated into large molecules of ~75 Kb and subjected to random-shotgun sequencing obviating the need for the cumbersome clone by clone approach of sequencing cDNAs through primer walking. Individual clone sequences are derived from the final sequence assembly by performing electronic digests prior to finishing. In the past year we have optimized this methodology to process ~4000 cDNA clones and in the past 4 months we have finished ~2400 FLcDNA clones from human, mouse and leukemic patients. As a part of this effort we have also developed informatics tools for assessing sequence quality to be applied to high-throughput finishing of clones. An annotation tool has also been developed for the rapid analysis of large batches of cDNA clone sequences to determine potential ORFs, exon/intron predictions, chromosome location, repeat content and homology searches in order to take cDNA clones beyond finishing toward gene discovery and disease characterization.

Program Nr: 223 from the 2001 ASHG Annual Meeting

The topographical expression map of chromosome 21 genes. *A. Reymond[1], V. Marigo[2], M. Yaylaoglu[3], A. Leoni[2], R. Lyle[1], C. Caccioppoli[2], C. Ucla[1], M. Guipponi[1], S. Banfi[2], G. Eichele[3], S. Antonarakis[1], A. Ballabio[2]*. 1) Univ. Geneva Medical School; 2) TIGEM, Naples; 3) Max Planck Institute, Hannover.

Down syndrome (DS), caused by the presence of an extra copy of human chromosome 21 (HC21), is the most common genetic cause of mental retardation with an incidence of approximately 1/700 live births. The sequence of the human chromosome 21 provided evidence for the presence of approximately 220 genes. An important challenge is now to understand how three normal copies of these genes are associated with the various DS phenotypes (mental retardation, congenital heart disease, early onset Alzheimers disease and an increased risk of leukemia). To define where HC21 genes exert their function and identify their possible role in the DS phenotype we have decided to perform a systematic analysis of the expression profile of their murine homologues . We collected 150 murine cDNA clones from several sources. To obtain a high resolution expression pattern several complementary methods were combined: (i) RT-PCR on a mouse cDNA panel of 12 adult tissues and 4 developmental stages; (ii) wholemount *in situ* hybridisation of E9.5 and E10.5 embryos and (iii) section *in situ*hybridisation of E14.5 embryos and of P7 brains. This set of whole embryos and sections corresponds to mid and late embryonic and foetal human periods, when most of the major organs and body regions are organised. Genes showing an interesting and/or restricted expression pattern were analysed further on appropriate sections at more developmental timepoints. 87% of the tested genes showed a clear expression pattern during murine development. In 37% of the cases expression was ubiquitous while 50% of the cDNAs showed a differential expression pattern in the embryonal tissues. The topographical catalogue of expression of the murine homologues of human chromosome 21 genes will be instrumental to the understanding of the pathogenesis of trisomy 21. Currently, approximately 100 genes have already been tested some of which display a pattern of expression relevant to the congenital heart disease and to the mental retardation observed in DS. The entire data set will be made available to the scientific community via a web site.

Program Nr: 224 from the 2001 ASHG Annual Meeting

Profiling Genomic Expression in Disorders of Epidermal Proliferation Identifies Egr1 as a Potent Regulator of Epidermal Growth. *M. Fang, S.A. Wee, H. Fan, G. Morrisey, S. Tao, Q. Lin, P.A. khavari.* Dept Dermatology, Stanford Univ Sch Medicine, Stanford, CA.

Epidermal hyperproliferation characterizes a number of skin disorders, including squamous cell carcinoma (SCC), basal cell carcinoma (BCC) and psoriasis. To understand the underlying gene expression programs responsible for the common and distinct features of these disorders, we performed gene expression profiling on a series of lesional and site matched normal control skin specimens from the same patients (n=4 per disorder) and verified these findings with protein expression via immunohistochemical analysis on 125 patients. Initial microarray hybridizations with 12,000 genes were extended to triplicate studies on 1,200 genes. Cluster analysis readily differentiated each disorder by pattern grouping, indicating that these disorders display disease-specific patterns of gene expression, which demonstrated the gene expression homogeneity in the order of psoriasis>BCC>SCC, consistent with psoriasis being a reactive disorder of normal tissue, BCC arising via defined pathways, and SCC arising via multiple mechanisms. PDGF-A was increased in all 3 disorders, suggesting the PDGF axis may be a common mediator of epidermal hyperproliferation. Egr1, a zinc finger transcription regulator, was induced strongly in psoriasis but completely absent in both BCC and SCC, raising the possibility that Egr1 may help differentiate benign and malignant proliferation. Consistent with this, Egr1 dramatically inhibited growth of proliferating normal human keratinocytes as well as HPV18 E6/E7 transformed cells and SCC-25 cells. Immunoblot analysis of cell cycle molecules demonstrated a dramatic decrease of Cdc25A phosphatase in Egr1 expressing keratinocytes, resulting in persistent phosphorylation of Cdk2. Targeted epidermal expression of Egr1 in transgenic mice was achieved, and Egr1 expressing epidermis appears resistant to PMA-induced hyperplasia. These data indicate that common disorders of epidermal hyperproliferation can be distinguished on the basis of global gene expression patterns and that differential expression of Egr1 may contribute to differences in biologic behavior of these conditions.

Program Nr: 225 from the 2001 ASHG Annual Meeting

Towards a complete understanding of active L1s in the human genome. *B.L. Brouha[1], R.M. Badge[2], E.M. Ostertag[1], A.F.A. Smit[3], J.V. Moran[2], H.H. Kazazian Jr.[1]*. 1) Genetics, University of Pennsylvania, Philadelphia, PA; 2) Dept. of Human Genet., Univ. of Mich., Ann Arbor, MI; 3) Inst. for Syst. Biol., Univ. of Wash., Seattle, WA.

L1 retrotransposons have played a major role in shaping the human genome. Previously, we estimated that of 3000-5000 full-length L1s, roughly 30-60 were retrotranspositionally active in a cell culture assay. These active elements were derived from the Ta subfamily of L1s, which contains roughly 200-250 full-length copies. We had previously found 10 active L1s with retrotransposition activity ranging from 1 event in 50,000 transfected cells to 1 event in every 30 cells ($L1_{rp}$). Here we used a genomic database approach to isolate 57 full-length Ta elements that are >99% identical to the active L1 consensus sequence and have 2 intact ORFs. PCR primers flanking the elements were used to amplify 10 of the 57 and clone 4 copies of each element to test in the retrotransposition assay. Of the 10 Ta elements, 8 had one or more clones that were retrotranspositionally active. The sequence of the 6 most active elements was closer to active L1 consensus (99.6-99.7%) than the sequence of the 4 weakly active or inactive elements (99.1-99.4%). Surprisingly, 2 of the 8 active new elements showed very high activity similar to that of $L1_{rp}$, suggesting that a number of human L1s are capable of very high frequency retrotransposition. The data allow us to make a second independent estimate of the number of active human L1s (70-100) that falls just above the range of the first estimate (30-60). In addition, 6 of the 8 active elements were present in 10-75% of genomes making them useful phylogenetic markers, whereas the 2 inactive elements were present in all genomes tested. This study nearly doubles the number of known active human L1 elements from 10 to 18. When this genomic analysis is complete, we will know the number, genomic location, activity level, and polymorphism information content of all active L1 elements in the sequenced human genome. The data provide further evidence that L1 elements continue to retrotranspose causing genomic expansion and insertional mutagenesis.

Program Nr: 226 from the 2001 ASHG Annual Meeting

Perinatal Genotoxicity Involving Genomic Instability and Molecular Cytogenetics in Human Immunodeficiency Virus (HIV-1) Infected HAART Treated Patients. *E.M. McGhee[1], J.P. O'Neill[2], W.L. Holzemer[1], J.A. Levy[3]*. 1) Dept. Community Health Systems, Univ California, San Francisco, CA; 2) Genetics Laboratory, Univ of Vermont, Burlington, VT; 3) Dept. Medicine, Univ California, San Francisco, CA.

Previous studies have shown that genetic changes are detected in peripheral blood CD4 positive T- lymphocytes from HIV+ patients treated with combination anti-viral drug regimens, that include dideoxy nucleoside analog viral reverse-transcriptase inhibitors (NRTIs). Failure to adhere to highly active antiretroviral therapy (HAART) can lead to subtheraputic drug levels which has been associated with increased genotoxicity and the development of drug resistance. We are currently investigating genomic instability and molecular cytogenetic effects of NRTIs in patients being treated with HAART, either for their own disease or to inhibit maternal fetal viral transmission. An unresolved issue is the potential genotoxic effects of HAART therapy in newborns. Recent studies found that 68% of infant cord blood leukocytes (n=22) were positive for AZT-DNA incorporation, with the positive values ranging from 22 to 451 molecules of AZT/106 nucleotides. In addition, a correlation was found between AZT-DNA incorporation and levels of intracellular AZT-triphosphorylated metabolite in cord blood leukocytes from 9 infants. Our data and others show that the HPRT mutant frequency in cord blood lymphocytes of unexposed children was half that of children exposed to the combination of AZT/3TC, and sequence analysis indicated that the formation of single-base transversion substitutions was >2-fold higher in the treated group. Overall, the data indicate that children of HIV-1-infected women, exposed in utero to nucleoside analog drugs, may sustain significant genotoxic insult and should therefore be subjected to long-term surveillance. Given this evidence for genetic damage in these newborns, consideration should be given to HAART induced, long term genomic instability. These studies can be performed in T-lymphocyte cultures. Further examination of genomic stability by cytogenetic analysis, flow cytometry, and microarrays will determine genomic and chromosomal instability.

Program Nr: 227 from the 2001 ASHG Annual Meeting

Natural history and complications in Proteus syndrome. *J.T. Turner[1], M.M. Cohen[2], L.G. Biesecker[1]*. 1) NHGRI, NIH, Bethesda, MD; 2) Dalhousie Univ, Nova Scotia, Canada.

Proteus syndrome (PS) is a rare overgrowth condition. Diagnostic criteria include sporadic occurrence, progressive course, and a patchy distribution. Additional criteria include A) connective tissue nevi, or B) (at least 2) epidermal nevi, disproportionate overgrowth, specific tumors, or C) (at least 3) dysregulation of adipose tissue, vascular malformations, and specific facial features. Twenty-six persons in our PS natural history study were reviewed to ascertain the range of severity and complications of PS. In addition, 65 cases of PS reported in the literature met the diagnostic criteria (duplicate cases were accounted for). Of these 91 total cases, 54 were male, 34 were female, and 3 were unspecified. Approximately 26% had mental retardation or developmental delay, which is greater than previously estimated. More than 25% of females were diagnosed as having cystic ovaries, ovarian or fallopian tube cystadenomas, or another tumor of the reproductive tract, whereas 22% of males developed testicular cysts or tumors. Pain was a common complaint often resulting from orthopedic problems or tumor-related discomfort. The majority underwent multiple orthopedic surgeries, as well as benign tumor and tissue resections. Hernia and hydrocele repairs were prevalent in males. Seizures were frequently but not exclusively found in individuals with hemimegalencephaly. Dermoids were the most common ophthalmologic complication. Tonsil hypertrophy was frequent, often accompanied by partial airway obstruction, as was cervical megaspondyly. Conductive and sensorineural hearing loss was frequently found in individuals with overgrowth of the external auditory canal. Decreased mobility and increased discomfort were associated with calcifications of the muscles, soft and fibrous tissues. Lethal complications include possible airway compromise and pulmonary embolism. The utilization of strict PS diagnostic criteria defines a population that is unlikely to be heterogeneous. Such strictly defined PS patients have a higher rate of complications than was previously estimated. We conclude that PS is more severe than the other overgrowth conditions that have similar manifestations.

Program Nr: 228 from the 2001 ASHG Annual Meeting

Intracranial hemorrhage in children with hereditary hemorrhagic telangiectasia. *M. Manning[1], T. Morgan[1], J. McDonald[2], C. Anderson[1], M. Ismail[1], F. Miller[2], A. Madan[1], P. Barnes[1], L. Hudgins[1].* 1) Stanford University, Stanford, CA; 2) HHT Clinic, Univ. of Utah, Salt Lake City, UT.

Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant vascular dysplasia which manifests as telangiectases or arteriovenous malformations (AVM) of the skin, mucosa and viscera. Epistaxis is the most frequent presentation, usually in late childhood. Visceral involvement includes pulmonary, gastrointestinal and cerebral AVMs, predominantly seen in adults. We describe 9 individuals with a family history of HHT who presented in childhood with intracranial hemorrhage secondary to cerebral AVM, including the first neonatal case in which there was molecular confirmation.

Case 1 was born limp, pale and without respiratory effort to a mother with known HHT. Postmortem exam revealed the presence of a venous angioma associated with hemorrhage. Linkage in this family, including the proband, was established to the endoglin gene on chromosome 9, confirming the diagnosis of HHT. Eight additional cases of infants and children presenting with intracranial hemorrhage are also discussed. All have positive family histories of HHT, although none of the cases were suspected to have the diagnosis prior to the hemorrhage despite symptoms of epistaxis and/or headaches in 5. One infant presented at 4 weeks of age, with the others presenting between 6 years and 16 years. Reports in the literature of children suspected of having HHT and presenting with symptoms secondary to cerebral AVM are few in number. Only 3 infantile (3 days to 6 weeks) cases of cerebral hemorrhage in families with known HHT have been reported.

HHT is very difficult to diagnose in childhood. The signs and symptoms are nonspecific and often show variable expressivity in families. These 9 cases that presented with cerebral AVM demonstrate the importance of consideration of this diagnosis in children with unexplained intracranial hemorrhage. Therefore, we suggest that clinicians consider HHT as a possible diagnosis in babies with this finding and stress the importance of obtaining a thorough family history.

.

Program Nr: 229 from the 2001 ASHG Annual Meeting

Genetic heterogeneity of McKusick-Kaufman syndrome (MKS) and Bardet-Biedl syndrome (BBS) phenotypes.

A.M. Slavotinek[1], L. Al-Gazali[2], R. Hennekam[3], C. Schrander-Stumpel[4], M. Orcana-Losa[5], A. Cantani[6], Q. Capellini[7], G. Neri[8], E. Zackai[9], L.G. Biesecker[1]. 1) NHGRI/NIH, Bethesda, USA; 2) United Arab Emirates University, UAE; 3) University of Amsterdam Hospital, Netherlands; 4) University of Maastricht, Netherlands; 5) University Reina Sofia, Cordoba, Spain; 6) University "La Spaienza", Rome, Italy; 7) Ospedale Ninetto Melli, Brindisi, Italy; 8) Universita Cattolica del S. Cuore, Rome, Italy; 9) Children's Hospital of Philadelphia, Philadelphia, USA.

MKS is an autosomal recessive (AR) syndrome comprising hydrometrocolpos (HMC), post-axial polydactyly (PAP) and congenital heart disease. BBS is an AR syndrome with retinitis pigmentosa, PAP, learning disability, obesity and renal and genital anomalies including HMC. Mutations in the MKKS gene at 20p12 cause both MKS and BBS phenotypes, but MKKS has so far been shown to be mutated in only 4% of unselected BBS patients. We hypothesized that BBS patients with clinical features more commonly described in MKS patients (for example, Hirschsprung disease) would have an increased frequency of mutations in MKKS. We sequenced the entire MKKS gene in 23 patients. Eight probands had BBS unlinked to the other known BBS loci, 8 females had MKS and HMC without satisfying the diagnostic criteria for BBS, 2 patients had BBS and HMC, 3 adult females had MKS, 1 male had BBS and Hirschsprung disease and 1 patient had BBS and hypothyroididsm. We found homozygous mutations in 2 of the unlinked BBS families (an 11 bp deletion causing a premature stop codon at amino acid 152 and G345E) and 1 mutation in the patient with BBS and Hirschsprung disease (a 4 bp insertion causing a premature stop codon at amino acid 327). The patient with BBS and hypothyroidism was heterozygous for A242S. The frequency of detected mutations in MKKS in our patients (13%) was three times higher than previously estimated for unselected BBS patients, suggesting that there may be phenotypic subgroups of BBS for whom sequencing of MKKS may prove useful. We also confirm the finding of single MKKS mutations in BBS patients, implying that MKKS may prove to be a modifier gene of the MKS and BBS phenotypes.*MKKSMKKSMKKSMKKSMKKSMKKSMKKSMKKS.*

Program Nr: 230 from the 2001 ASHG Annual Meeting

Clinical and molecular heterogenity for Madelung deformity of childhood. *I.A. Glass[1,2], S. Flanagan[2], C.F. Munns[2], M.T. Hayes[2], D. Vickers[2], G. Rappold[3], J.A. Batch[2], V.J. Hyland[2].* 1) University of Washington; 2) University of Queensland; 3) University of Heidelberg.

Madelung deformity is a painful and disabling abnormality of the forearm, due to a dyschondrosteosis of the distal radial physis. Leri-Weill dyschondrosteosis (LWS), characterised by bilateral Madelung deformity (BMD), disproportionate stature and mesomelia, is due to haploinsufficiency of the gene encoding SHOX. Identification of sporadic, normal statured BMD patients suggested genetic heterogeneity for dyschondrosteosis. We evaluated a surgical cohort of familial and sporadic BMD subjects, and their families. In our cohort, mutations of SHOX were present in 12/18 BMD probands, encompassing 6 FISH detectable deletions, 3 point mutations (R195X, Y35X, and IVS2-1G>A),of which 2 are novel and three 5'rearrangements, identified by segregation of PAR1 markers. Analysis of SHOX expression by RT-PCR in bone marrow fibroblasts, confirmed SHOX haploinsufficiency in 2 probands harboring the IVS2-1G>A and hemizygousity at the PAR1 marker, DXYS233, respectively. Histopathological sectioning of resected radial physeal tissue in 2 LWS cases demonstrated that beyond the zone of dyschondrosteosis, a more generalized disturbance of growth plate structure and organisation was present. From extended family studies (n=116, we determined LWS subjects (n=34) to be significantly shorter at birth, through childhood and at final height, with disproportionate stature and a greater arm span reduction, compared to unaffected 1st degree relatives (n=48) and probands with wild type SHOX alleles (n=5). BMD probands with wt SHOX alleles were sporadic (5/6), apart from one family. Overall, haploinsufficiency of SHOX appears to account for about 18cm of final height in females and 15cm of final height in males, with the most marked impact being in pubertal females. These results suggest that SHOX is a statural gene of major effect, with actions that begin in utero and continue until attainment of final height. We have confirmed marked variability of LWS from our family based ascertainment/investigations and demonstrated that clinical and molecular heterogeneity exists for BMD/dyschondrosteosis of childhood.

Program Nr: 231 from the 2001 ASHG Annual Meeting

Beta-adrenergic-antagonist and melatonin administration resets biological clock, improves day behavior and restores sleep in the Smith-Magenis syndrome. *H. De Leersnyder, M.C De Blois, J.L Bresson, A. Mogenet, J.C Souberbielle, D. Sidi, A. Munnich.* Dept Genetics and Pediatrics, Hosp Necker, Paris, France.

Smith-Magenis syndrome (SMS) is a multiple congenital anomaly syndrome caused by an interstitial deletion of chromosome 17p11.2. Common features include dysmorphism, hyperactivity, and sleep disturbances. This behavioral phenotype has been ascribed to an inverted rhythm of melatonin: hyperactivity and naps occur when melatonin rise at midday, SMS children display a sleep phase advance with sleep attacks (6pm), early sleep onset (8.30pm), frequent awakenings and early sleep offset (5am) when melatonin is low during the night. Considering that beta-blockers decrease melatonin release via specific inhibition of adrenergic beta1-receptors, these results prompted us to test the benefit of beta-blockers and melatonin administration in resetting the day/night rhythm. We studied 10 SMS children(4 to 19 years) through consultations, sleep and day diaries, 1 month actimetry, and 48 hours hospitalizations for EEG recording and plasma melatonin work up. Children were given beta- adrenergic antagonist (10mg/kg/day) in a single morning dose for 12 months and prolonged-release melatonin (6mg/day) was given in a single evening dose in the last 6 months. Following beta-blockers administration, mean melatonin levels fell from 155 pg/ml to 8 pg/ml at midday. Day behavior improved: naps and sleep attacks disappeared, children were less hyperactive and concentration increased, home and social behavior improved. Adding melatonin in the evening reset circadian rhythm of melatonin: plasma melatonin remained low during the day, rised at 10pm (2190 pg/ml) , remained high until 2am (1500 pg/ml), and decreased until 6am (200 pg/ml). Actigraphy recordings were correlated with day and sleep diaries: mean sleep onset was delayed at 10pm, children no more woke-up during the night, sleep offset was at 7am, and mean duration of sleep was 9 hrs. In conclusion SMS children present an inversion of the circadian rhythm of melatonin. Morning beta-adrenergic antagonist and evening melatonin administration restored normal melatonin circadian rhythm, improved day behavior and restored normal sleep habits in SMS.

Program Nr: 232 from the 2001 ASHG Annual Meeting

D/G galactosemia: To treat, or not to treat? *R.E. Schnur[1], C. Howard[1], K. Annable[1], L. Wells[1], G. Berry[2], S. Segal[2], M. Palmieri[2]*. 1) Cooper Health System, Robt Wood Johnson Med Sch, Camden, NJ; 2) Children's Hosp of Phila, PA.

Treatment of D/G galactosemia is controversial. There are few formal studies evaluating galactose metabolites, liver dysfunction, cataracts, premature ovarian failure, and cognitive defects in these individuals. Galactose 1-phosphate uridyl transferase (GALT) activity is reduced to ~25% of normal in this biochemical phenotype. Most individuals are compound heterozygotes for a classic galactosemia mutation (Q188R or S135L) and the less severe Duarte allele (N314D). From 5/98-6/01, 22 infants with D/G galactosemia were identified after having positive newborn screens. Confirmatory evaluations included physical examination (usually at 1-3 weeks of age), GALT quantitation, isozyme analysis, and measurement of metabolites-galactose 1-phosphate (G1P) and urine galactitol. Most also had DNA analysis for common mutations. Seven infants had elevated total galactose levels on the first screen. At the initial evaluation, four had significant hyperbilirubinemia, with total bilirubin ranging from 6 (at 23 days)-17.3 mg/dl (at 10 days). Liver spans were assessed by percussion; ten were 5-5.5 cm and two were >6 cm. 2/16 newborns had a slight elevation of urine galactitol. Another 2/16 had elevated G1P levels (3.8 and 20.6 mg %). Notably, both were still drinking breast milk when testing was done. Infants were treated with galactose-restriction until 1 year of age. They were then given an ad lib 7-10 day milk "challenge" followed by re-analysis of galactose metabolites. To date, nine children have had their milk "challenge" at 12-14 months. Clinically, none had hepatomegaly or jaundice when examined. However, 6/9 "failed" the first challenge biochemically. All six had increased urine galactitol; one also had increased G1P. The diet was re-instituted; subsequent "challenges" were performed at three-month intervals. One child did not "pass" the test until she was 19 months old. Our observations suggest that children with D/G galactosemia often have physical and/or biochemical galactose intolerance and should probably be treated until ³ 12 months of age, until they pass a milk challenge. More studies are needed to evaluate the long-term sequelae of this variant.

Program Nr: 233 from the 2001 ASHG Annual Meeting

Fetal cells in the maternal liver: an opportunity for "reverse" stem cell therapy. *K.L. Johnson[1], H. Stroh[1], L. Bailen[2], M. McDonnell[3], J.L. Nelson[3], D.W. Bianchi[1].* 1) Dept Pediatrics, Div Genetics, New England Med Ctr/Tufts Univ, Boston, MA; 2) Dept Medicine, Div Gastroenterology, New England Med Ctr/Tufts Univ, Boston, MA; 3) University of Washington, Seattle, WA.

We are studying the role of fetal cell microchimerism in the etiology of a number of diseases with increased prevalence in women, such as systemic sclerosis. Prior experiments have shown that many fetal cells, as documented by the presence of X and Y chromosome FISH signals in interphase cells obtained from paraffin-embedded tissue, are present in the spleen and tissues clinically affected by the disease process (Johnson et al., Arthritis Rheumatism 2001; in press).

In preliminary work, we performed FISH analysis on liver specimens from women with primary biliary cirrhosis (PBC), as this disease almost exclusively occurs in middle-aged females. We did not find striking evidence of fetal cell microchimerism in women with PBC who had previously delivered a male child. However, in a control subject with hepatitis C, a very high number of male cells were detected (5 slides analyzed, average > 400 male cells per cm^2 tissue). These cells were morphologically indistinguishable from the surrounding female liver tissue. Subsequent PCR amplification of the male cells using short tandem repeat (STR) sequences suggested that these cells originated from a pregnancy that spontaneously miscarried.

We have now studied archived liver samples from an additional 12 women severely affected with hepatitis C, who have undergone liver transplantation. Male cells were detected in 7 of 10 samples studied, with the remaining 2 samples having inadequate hybridization efficiencies. Thus, our preliminary evidence suggests that fetal stem cell microchimerism may be common in women affected with hepatitis C. This research suggests that fetal stem cells normally acquired as part of pregnancy or delivery may have the capacity to repair diseased tissue. Approaches to gene therapy using endogenous stem cells should also consider the potential benefits of using fetal cells in the mother's body.

Program Nr: 234 from the 2001 ASHG Annual Meeting

Efficacy and safety of prolonged enzyme replacement therapy for Fabry disease. *R. Schiffmann[1], J.B. Kopp[2], D.F. Moore[1], T. Weibel[1], G. Charria[1], R.O. Brady[1]*. 1) Developmental and Metabolic Neurology Branch/NINDS, NIH, Bethesda, MD; 2) Metabolic Diseases Branch/NIDDK, NIH Bethesda, MD.

Fabry disease is an X-linked recessive disorder caused by a deficiency of lysosomal a-galactosidase A and accumulation within susceptible cells of the glycosphingolipid globotriaosylceramide (Gb3). Clinical manifestations include severe painful neuropathy and progressive renal deterioration with premature death. No effective treatment has existed. We recently demonstrated therapeutic efficacy and safety of enzyme replacement therapy (ERT) for Fabry disease in a 6-month, randomized, double-blind, placebo-controlled trial (Schiffmann, et al, 2001). Here we report the results of an open-label extension of the initial ERT trial in which all 25 hemizygous patients received 40-minute infusions of 0.2 mg/kg of Replagal^{TM} (Transkaryotic Therapies, Inc., Cambridge, MA) every two weeks for an additional year. Outcome measures included renal function, neuropathic pain measured while off analgesics using the Brief Pain Inventory, sensory function, and Gb3 levels in plasma and urinary sediment. In the patients who crossed-over from placebo to Replagal (n=10), glomerular filtration rate significantly increased (78 to 95 ml/min/1.73 m^2, p=0.025), and pain-at-its-worst scores on a 0-10 scale decreased (from 6.9 to 4.5, p=0.02). Gb3 in plasma (10.9 to 5.0 nmol/mL, p=0.002) and in urine sediment (2494 to 455 nmole/g creatinine/24h, p=0.002) declined significantly. The patients who were initially treated with Replagal (n=14) had stable inulin clearance (72 to 74 ml/min/1.73 m^2) and additional decreases of Gb3 in plasma (12.1 to 6.2 nmol/mL, p<0.001) and in urinary sediment (2368 to 812 nmole/g creatinine/24h, p<0.001). Cold and warm detection thresholds significantly improved in the hand and in the foot. Body weight significantly increased in the two groups. Replagal infusions were very well tolerated allowing the transfer of patients to home therapy at the end of the study. We conclude that ERT in Fabry disease is safe, has widespread therapeutic effects, and based on the stabilization or improvement of renal function, is expected to improve the overall survival of patients with this disorder.

Program Nr: 235 from the 2001 ASHG Annual Meeting

Mesenchymal Stem Cell-Based Therapy for Type A Niemann-Pick Disease: Studies in the Mouse Model. *E.H. Schuchman, J. Carter, H.-K. Jin.* Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY.

Types A and B Niemann-Pick disease (NPD) result from the deficient activity of the lysosomal enzyme, acid sphingomyelinase (ASM). Type A NPD is characterized by early onset neurodegeneration that leads to death by ~3 years of age. A mouse model of NPD (ASMKO mice) has been constructed that develops progressive neurodegeneration leading to death by about 6-8 months of age. Previous studies in this mouse model have demonstrated that residual ASM activities as low as ~1-2% of normal in the brain can significantly alter the progression of CNS disease, and that activities within ~8% of normal can completely prevent it. To evaluate the potential of bone marrow-derived mesenchymal stem cells (MSC) to engraft and treat the CNS disease in ASMKO mice, we retrovirally-transduced MSCs from GFP-expressing transgenic mice to overexpress human ASM, and then transplanted ~10E5 transduced cells into the hippocampus and cerebellum of young ASMKO mice. We found that the injected cells could migrate throughout the CNS and survive up to at least 8 months post-transplant. About 20% of the transplanted cells also became positive for neural markers. Following transplantation about half of the treated mice had improved rotorod performance compared to untreated mice (although far below normal), and survived up to at least 10 months. Histological analysis of the CNS at various times post-transplantation revealed dramatic effects of this gene therapy procedure on the CNS lipid storage and Purkinje cell numbers. We conclude that bone marrow-derived MSC may be a very useful cell type for cell-based therapies of Type A NPD and other neurodegenerative lysosomal storage disorders, particularly since this same cell type could also be used to treat the visceral organ complications associated with these diseases.

Program Nr: 236 from the 2001 ASHG Annual Meeting

In vivo Correction of a Mouse Model of Hereditary Tyrosinemia Type I by the Sleeping Beauty (SB) Transposon.

E. Montini[1], M. Noll[1], N. Morcinek[1], A. Major[2], M. Finegold[2], M. Kay[3], M. Grompe[1]. 1) Dept. of Medical & Molecular Genet, Oregon Health Sciences Univ, Portland, OR; 2) Dept. of Pathology, Texas Children's Hospital, Baylor College of Medicine, Houston, TX; 3) Dept of Pediatics, Stanford University School of Medicine, Stanford, CA.

The *Sleeping Beauty* element, a reconstructed version of Tc1/mariner-like transposon from fish, can transpose from plasmid DNA into the chromosomal DNA of a wide variety of eukaryotic cells. Recently, this transposon technology has been successfully applied for in vivo gene transfer into the liver in a mouse model of hemophilia B. Because the use of naked DNA to achieve stable gene expression is highly desirable we tested the ability of this system to cure a mouse model of Hereditary Tyrosinemia Type 1 (HT1), deficient in fumarylacetoacetate hydrolase (FAH). A full length human FAH cDNA, under the control of the synthetic promoter SR-a was cloned into the transposon vector pT-MCS (pT-SR-a-FAH). FAH-/- mice were injected with a combination of the plasmid pT-SR-a-FAH and pCMV-SB (encoding the SB transposase) or pCMV-mSB as negative control (encoding a mutated SB transposase). The treated mice were taken off the protective drug NTBC to start positive selection of the FAH+ hepatocytes (HCs). After removing NTBC and 8 weeks of in vivo selection the livers of all transposon injected FAH- mice (7/7) appeared macroscopically healthy. Histological liver sections stained with FAH antibody detected large, confluent areas of FAH+ hepatocytes. Liver function tests were completely normalized indicating functional correction of HT1. In order to quantitate the number of stable of integrations a cohort of transposon injected mice was analyzed 20 days after initiation of selection. At this stage stabily FAH positive; hepatocytes have formed small, readily countable nodules. We estimate that ~ 1-1.5 % of hepatocytes were stabily corrected by a single systemic injection of the FAH-transposon. HCs from repopulated livers mice were also serially transplanted and rescued a second generation of mutant mice. Southern blot analysis to study the clonality of repopulation as well the nature of integration events are currently ongoing.

Program Nr: 237 from the 2001 ASHG Annual Meeting

Licensure of Genetic Counselors: Success in Utah. *C.E. Miller[1], B. Baty[1], L. Berkheim[1], M. Cantwell[1], K. Dent[1], J. McDonald[1], L. Mensching[1], J. Palumbos[1], L. Pho[1], C. Solomon[1], V. Venne[1], B. Hafen[2], E. Rosenthal[3], A. Poss[4].* 1) Univ Utah, UT; 2) LDS Hospital, SLC, UT; 3) Univ California at San Diego; 4) Harvard Vanguard, Boston, MA.

Licensure of genetic counselors was attempted in Utah in hopes of serving multiple purposes: 1)Legitimatize the profession 2) Provide public protection 3) Aid negotiations with Medicaid and commercial insurers 4) Ultimately petition the AMA CPT committee for appropriate codes for genetic counselors. State Licensure remained elusive for genetic counselors nationally until California achieved such in the fall of 2000. In Utah, support for licensure was elicited from the director of the Department of Occupational and Professional Licensure (DOPL). Members of the Utah Senate and the House of Representatives were chosen to sponsor the bill. The Utah Medical Association, UMA, was contacted to encourage their support and endorsement. To receive such, UMA requested completion and submission of a licensure criteria questionnaire. Utahs Sunrise Law requires professions applying for licensure to answer a 52 question document and make a presentation at a hearing of the Sunrise committee (3 Senators, 3 House of Representatives, and 6 Governor appointees. Genetic counselors, MD geneticists, and patients testified before the committee. Next the genetic counselors licensing bill, Senate Bill 59 (SB59), was drafted by an attorney in the Office of Legislative Research and General Counsel with the assistance of Utah's genetic counselors. A bullet sheet of key points was created for the Senator sponsoring SB59 for her presentation to the Senate committee, Senate, and House Committee. Genetic counselors, medical geneticists, and prior patients testified before the House and Senate Committees. The House sponsor presented SB59 on the House floor and it was passed out successfully. All through the legislative process, from the Sunrise Committee to the Senate and House, genetic counselors, geneticists, supporting MDs, and patients, helped lobby key politicians through emails, phone calls, and personal meetings. Finally SB59 was signed into law by Utah's Governor on March 15th 2001. Utah's successful pursuit of licensure sends a powerful message: Even the most conservative states and those with few practicing genetic counselors can succeed in this endeavor.

Program Nr: 238 from the 2001 ASHG Annual Meeting

Patients' ethical views: USA, Germany, France. *D.C. Wertz[1], I. Nippert[2], G. Wolff[3], S. Ayme[4].* 1) Univ Massachusetts,Shriver Div, Waltham, MA; 2) University of Muenster, Germany; 3) University of Freiburg, Germany; 4) INSERM, Villejuif, France.

We surveyed patients at 12 genetics clinics in USA , 2 in Germany, 5 in France. 476(67%) in USA, 593(65%) in Germany, and 394(51%) in France returned anonymous questionnaires. Over 9/10 in USA and Germany were women. Majorities (US 75%, Germany 76%, France 88%) thought warning relatives takes precedence over patient confidentiality. About half (US 53%, Germany 42%, France 55%) thought spouses had automatic rights to information, without consent; 44%,32%, 41% favored automatic access for blood relatives. 61%,34%,45% would test children for predisposition to Alzheimer. 60%,48%, 45% said patients had a right to any service they could pay for; 69%, 46%, 48% thought refusal was denial of rights. Most believed (86-88%) in rights to referral within their country; fewer (50%, 29%,33%)favored it outside the country. Most (87%,82%,70%) approved prenatal paternity testing for a woman with 2 male friends. C. Privacy. There was universal distrust of insurers and employers; most (US 96%, France 92%) would tell a school system a diagnosis of XYY. 20%,11%,6% would protect confidentiality of a bus driver at high risk for heart attack. Most favored DNA fingerprinting for people convicted of (83-97%) or charged with (71-82%) serious crimes, armed forces (60-86%), but not passport applicants (50%,10%,12%) or newborns (73%,31%,47%). Majorities (64%,49%,70%) thought people should know genetic status before marriage: fewer (31%,5%,29%) thought states should require carrier tests. Most (80-93%) thought women at high risk should have PND; fewer (21%,43%,67%) thought they should abort. 44%,48%,77% said having a child with a disability was unfair, if the birth could be prevented; 26%, 27%, 56% said it was socially irresponsible. 21% in US and 18% in France said laws should require sterilization for a blind woman on welfare. About 30% fewer in US would abort for each of 24 genetic conditions than in Germany . 26%,10%,20% would use preconception sex selection. On most issues, patients views differed from geneticists'.

Program Nr: 239 from the 2001 ASHG Annual Meeting

Prader-Will syndrome: Impact on sibling relationship qualities. *E.L. Burner[1], S. Heeger[1], J. Robinson[1], M. Warman[1], E. Eichler[1], S. Cassidy[2], A.L. Matthews[1].* 1) Genetics, Case Western Reserve Univ, Cleveland, OH; 2) Genetics, UC Irvine Medical Center, Irvine, CA.

While the literature probing the impact of chronic illness on family systems, and siblings specifically, continues to expand, there are no studies on how siblings are affected by living with a brother or sister with Prader-Willi syndrome (PWS). As an initial effort to investigate this situation, we focused on specific aspects of the sibling relationship hypothesized to either resemble or differ from sibling relationships involving developmental disability. Relevant factors contributing to siblings' feelings towards having a brother or sister with PWS were also investigated. A total of 61 participants ages 10-46 were recruited, with all participants having at least one sibling with PWS. Siblings were asked to complete the Sibling Relationship Questionnaire (SRQ), as well as to respond to several open-ended questions aimed at exploring additional aspects of the sibling relationship. Previously published studies utilizing the SRQ comprised the comparison group. Analyses of siblings' responses on the SRQ revealed participants' relationship with their PWS sib as being characterized by high levels of affection, low levels of intimacy, and discriminate power and status in the direction of the older healthy sibling. Interestingly enough, participants also noted that their sibling relationship was more conflictual than that described in studies between siblings and brothers or sisters with a developmental disability. While siblings reported that their mother showed slight partiality towards their brother or sister with PWS, they also indicated that there was either no difference in the amount of attention their father gave them or that their father treated them somewhat better compared to their PWS sib. The results suggest that these siblings may benefit from learning conflict-resolution skills, as well as being encouraged to share their experiences with other siblings in similar situations. Furthermore, parents of these siblings may find it helpful to switch their traditionally circumscribed roles to balance the treatment and attention given to their children.

Program Nr: 240 from the 2001 ASHG Annual Meeting

Adults with Marfan Syndrome: Sexual Functioning and Reproduction. *B.B. Biesecker[1], K.F. Peters[2], F. Kong[3]*. 1) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Dept of Biobehavioral Health, Pennsylvania State University, University Park, PA; 3) Westat Research, Inc. Rockville, MD.

As individuals with Marfan syndrome are increasingly diagnosed prior to childbearing, there is opportunity to study factors related to the diagnosis that influence their reproductive planning. Data will be presented from an exploratory study of 174 adults with Marfan syndrome regarding their reproductive plans. A majority of survey respondents were Caucasian and well educated. Fifty eight percent were female and 64% were members of the National Marfan Foundation. Sixty percent of the cohort reported difficulties with sex drive. Age (50+ yrs), marital status, striae, back pain, and low quality of life were each independently correlated with a lack of sex drive. Forty percent of the respondents had children, with 23% having one or more child affected with Marfan syndrome. Approximately 62% of the total cohort agreed that having Marfan syndrome has significantly affected their decisions about having children. Age at diagnosis, mitral valve prolapse, and the view that Marfan syndrome has adverse consequences on ones life were each independently correlated with the perception that being affected had influenced their reproductive plans. Of the total cohort, 69% reported that they were interested in prenatal testing for Marfan syndrome. Both clinical features and psychosocial issues contribute negatively to affected adults reproductive decision-making and sexual well-being. Genetics professionals are ideally positioned to address concerns surrounding reproduction with Marfan syndrome patients and to refer those with significant sexual or reproductive concerns for further evaluation and management.

Program Nr: 241 from the 2001 ASHG Annual Meeting

Affected Parents with a 22q11.2 Deletion: The Need for Basic and Ongoing Educational, Health, and Supportive Counseling. *M. Tonnesen[1], D.M. McDonald-McGinn[1], K. Valverde[2], E.H. Zackai[1]*. 1) Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Genetic Counseling Training, Beaver College, Glenside, PA.

The 22q11.2 deletion has been identified in the majority of patients with DGS, VCFS, CTAF & some cases of autosomal dominant Opitz/GBBB & Cayler cardiofacial syndrome. Approximately 10% of cases are familial with the majority of parents identified only following the diagnosis in a more severely affected child (McDonald-McGinn et al., 1999). Most patients have a learning disability or borderline intelligence (Moss et al., 2001), making parents affected with the deletion similar functionally to adults with low average intelligence. With this in mind, we developed a written questionnaire to assess differences in parental medical knowledge & compliance between familial & de novo cases of the deletion. Following genetic counseling, 20 affected parents & 50 sets of unaffected parents identified from our existing cohort (N = 370) received questionnaires. Only 27% of parents affected with the deletion correctly reported their recurrence risk as compared to 50% of unaffected parents. Similarly, 27% of parents with the deletion understood their child's recurrence risk compared to 90% of unaffected parents. Only 36% of affected parents believed that they were very well or moderately educated about the deletion as compared to 78% of unaffected parents. Regarding medical compliance, we found a lack of ongoing subspecialty care for children with familial cases of the deletion as compared to non-familial cases. Many had never seen immunology (60%), child psychology (50%), otolaryngology (40%), and plastic surgery (20%) compared to 13%, 3%, 5%, and 8% of de novo cases, respectively. These findings suggest affected parents are less knowledgeable about the deletion and often non-compliant. Because of this population's similarity to adults with other forms of learning differences, it may be necessary to redirect the traditional genetic counseling approach to a more psychosocial focus (Finucane, 1998). Awareness of these facts should allow genetic professionals to better communicate with this under-served population.

Program Nr: 242 from the 2001 ASHG Annual Meeting

Preliminary findings from the REVEAL Study: Genetic risk assessment and counseling for Alzheimer's disease.

T. Brown[1], S. LaRusse[2], M. Barber[3], N. Relkin[2], P. Whitehouse[3], L. Farrer[1], L. Cupples[1], S. Post[3], L. Ravdin[2], H. Katzen[2], D. Cisewski[1], S. Sami[3], J. Davis[2], A. Sadovnick[4], K. Quaid[5], J. Woodard[6], R. Green[1]. 1) Boston U. School of Medicine; 2) Cornell U; 3) Case Western U; 4) U. British Columbia; 5) Indiana U; 6) Chicago Medical School.

The REVEAL Study (Risk Evaluation and Education for Alzheimer's disease) is an NIH funded project (R. Green, PI) designed to examine the impact of providing risk assessment, including Apolipoprotein E (APOE) genotyping, to asymptomatic adult children of persons with Alzheimer's. To date, 217 REVEAL participants have been enrolled, 149 have attended the introductory educational session, 107 have proceeded through neuropsychology screening and blood draw, 86 have received results disclosure and 66 have been seen for one or more follow-up visits. Individuals who have enrolled but have chosen not to continue through the results disclosure step have cited several reasons for doing so including insurance concerns, lack of free time and changing their mind about wanting to learn risk or genotype information. Of those seen for results disclosure, 62 were randomized to receive a personalized risk estimate based on both family history and APOE genotype information (Study Group). The remaining 24 participants were randomized to receive a risk estimate based on family history information only (Control Group). Of the Study Group participants who received APOE results, 28 had a 3/3 genotype, 27 had 3/4, 4 had 2/3, 2 had 4/4 and 1 had 2/4. Participants reactions to personalized risk estimates were varied and included being upset, having preconceived risk beliefs confirmed and/or feeling reassured by the information given. Control Group members were sometimes frustrated by not receiving APOE genotype results. Outcome variables include measures of anxiety, depression and satisfaction with the risk assessment and counseling protocol, as well as real-world decisions to change retirement planning or insurance coverage. Results of preliminary analyses and selected case reports will be presented. REVEAL is the first study of its kind, and results will inform policy debates and future guidelines about the advisability of such disclosures.

Program Nr: 243 from the 2001 ASHG Annual Meeting

Taking the Challenge: Finding Recurrence Risks in Idiopathic Mental Retardation. *J.S. Collins[1], A.F. Nave[1], G.A. Satten[2], R.E. Stevenson[1]*. 1) Greenwood Genetic Center, Greenwood, SC; 2) Centers for Disease Control and Prevention, Atlanta, GA.

Since causation is unknown in approximately 50% of all cases of mental retardation (MR), there is a frequent need for recurrence risk figures in genetic counseling. Currently clinicians and counselors rely on studies conducted over 25 years ago in counseling families of affected subjects. In 1998, a survey by Crow and Tolmie found that clinical geneticists quote recurrence risks anywhere between 1% and 25% for idiopathic MR. They called for further studies to incorporate clinical features and newer diagnostic techniques in risk calculations.

To date, we have gathered information on probands (269 M, 183 F; 171 black, 194 white, 2 Hispanic, 85 unknown) from 452 families studied by the Greenwood Genetic Center. These individuals had an an average IQ of 41.1 and average head circumference at the 43rd percentile. One hundred and twenty four had seizures, 84 had a form of psychosis, and 31 had autism. Seventy five percent have been karyotyped, 24% have had cytogenetic fragile X studies, 43% have had FMR1 testing, 33% have had plasma amino acids analysis, and 14% have had urine metabolic screening. All of these tests on the probands were normal.

In order to qualify for the study, probands must have at least one younger sibling, older than 6 years of age. In these families there were 502 male and 468 female subsequent siblings. Of these, 107 (21%) brothers and 67 (14%) sisters also had MR. Recurrence in blacks was 18.3%, and recurrence in whites was 17.0%. Individuals with IQs of £20, 21-35, 36-50, and 51-70 had recurrence risks of 9.8%, 13.6%, 20.6%, and 22.7%, respectively. Additional probands and family information will be added to these preliminary results, and the data will undergo more detailed analyses.

.

Program Nr: 244 from the 2001 ASHG Annual Meeting

Genetic factors in the risk of developmental delay and malformations in children and adults exposed to antiepileptic drugs in utero. *J.C. Dean[1], H. Hailey[1], S.J. Moore[2], D. Campbell[1], P. Turnpenny[3], D. Lloyd[1], J. Little[1].*
1) University of Aberdeen Medical School, Aberdeen, Scotland; 2) Medical Genetics Program, Memorial University of Newfoundland, St John's, Newfoundland, Canada; 3) Clinical Genetics Service, Royal Devon and Exeter Hospital, Exeter, England.

Between 5 and 14% of children born to mothers taking antiepileptic drugs (AEDs) such as carbamazepine, phenobarbitone, phenytoin, and valproate have congenital malformations. Prospective data concerning the risk of developmental delay are scarce. The relative roles of familial factors and AED teratogenesis are controversial. We ascertained a group of mothers who took AEDs in pregnancy and delivered at Aberdeen Maternity Hospital in the past 20 years. 292 children (mean age 10 years) from 150 families were assessed. Mothers took carbamazepine alone in 23% of pregnancies, phenobarbitone in 21%, valproate in 16%, phenytoin in 8%, other monotherapy in 2%, polytherapy in 17% and no therapy in 13%. 32 (13%) exposed children have congenital malformations (2/38 not exposed), but 56 (26%) have developmental delay (4/38 not exposed). Amongst those exposed to monotherapy, malformation was most frequent for phenytoin (17%) and developmental delay most frequent following valproate or phenytoin (34% in both groups). Of those with developmental delay, 16 have a family history suggesting a single gene or multifactorial contribution. When these cases are excluded, 43 (19%) have developmental delay attributable to fetal anticonvulsant effects. Cases with malformation or developmental delay were drawn from 64 families, there were 20 recurrences. After excluding cases with a family history of non-AED related developmental disorder, the recurrence risk using Weinberg's proband method is 41%. This study supports the view that some AEDs may cause developmental delay and learning disability in children exposed in utero, and that there may be a genetic susceptibility.

Program Nr: 245 from the 2001 ASHG Annual Meeting

Intrusive Ideation About Cancer Risk Before and After BRCA1/2 Mutation Testing. *K. Hurley[1], H. Valdimarsdottir[1], K. Brown[1], J. Rispoli[1], L. Stravinski[1], J. McGlynn[1], D. Grant[1], C. Eng[1,2]*. 1) Mt. Sinai Sch. of Med., New York, NY; 2) Baylor Coll. of Med., Houston, TX.

Intrusive ideation (repetitive, unwanted thoughts) about one's cancer risk is a common and distressing psychological symptom among women with a family history of breast cancer. Negative outcomes associated with intrusive ideation about cancer risk include nonadherence to cancer screening, interest in prophylactic surgery in excess of actual risk, decreased quality of life, and depression. We examined levels of intrusive ideation among Ashkenazi Jewish women before they underwent counseling about genetic testing for BRCA1/2 mutations, and after they were counseled about their results.

55 women (age X=50.6) seeking genetic testing for breast/ovarian cancer risk completed pre-counseling and post-notification questionnaires. Regression analysis showed that post-notification intrusive ideation was predicted by baseline intrusive ideation($p<.0001$), past history of cancer($p<.01$), and mutation status($p<.05$). 30% of mutation carriers, 26.7% of those with inconclusive/negative results and 0% of true negatives had post-notification intrusive ideation scores in the clinical range (comparable to those seeking treatment for post-traumatic stress disorder). Approximately 1/3 of the sample reported an increase in intrusive ideation after receiving their results, regardless of mutation status. Affected women were more likely to report an increase in intrusive ideation after receiving inconclusive results than unaffected women($p<.05$). Finally, among a subset of the entire sample, although 100% were very/extremely satisfied with information received, and 77.8% found counseling very/extremely helpful in making medical decisions, only 22.3% felt that counseling fully met their need for emotional support.

Our data indicate an unmet need for psychological services among women undergoing testing for BRCA1/2 mutations, particularly affected women who receive inconclusive results. Addressing these needs will help women make effective use of educational and decision-making gains achieved by genetic counseling to promote long-term emotional adjustment and adherence to risk-reduction behaviors.

Program Nr: 246 from the 2001 ASHG Annual Meeting

Developing cancer genetics educational materials for African-Americans. *B.J. Baty, A.Y. Kinney, S. Ellis, A. Wiley.* Univ Utah Health Sciences Ctr, Salt Lake City, UT.

The goal of this project was to develop culturally sensitive education materials for an African-American kindred in Louisiana with a known *BRCA1* mutation. A team of two genetic counselors, an oncology nurse/epidemiologist, a nursing student, an African-American study coordinator and an African-American artist assembled and revised educational materials. Materials were pilot tested using four African-American focus groups varying in socioeconomic status, including one group of men. Although individuals were recruited in Utah and Louisiana through the media and area churches, personal networking of the study coordinators proved more effective. The audio taped focus groups consisted of the proposed educational presentation made by the genetic counselor, followed by a feedback session led by an African-American facilitator. Using NUD•IST software, qualitative analysis of the focus group transcripts was done to identify important themes and specific suggestions. The educational materials were revised in response to the participants' suggestions. The products included a flip chart for use in educational sessions and a pamphlet. Focus group participants recommended that technical detail be reduced substantially, and that information be personalized and made relevant to the lives of the participants. Other critical themes included the importance of building trust in the medical system and avoiding usage of words and images that have negative associations in the African-American community. Strategies that were successful included non-technical images to explain genetic concepts, clip art images to energize and personalize word slides, vibrant color, identifiably African-American figures, and the development of themes highly relevant to many African-Americans. The input from focus group participants was critical in making culturally sensitive changes to educational materials prepared for use with African-American families. Their use in an ongoing study offering *BRCA1* counseling and genetic testing to a large, rural Louisiana-based kindred will provide additional feedback about the effectiveness of the culturally tailored genetic education and counseling materials.

Program Nr: 247 from the 2001 ASHG Annual Meeting

Hierarchical linkage disequilibrium mapping of a susceptibility gene for Crohn's disease (CD) to the cytokine cluster on chromosome 5. *J.D. Rioux[1], M.J. Daly[1], M. Silverberg[2], K. Lindblad[1], H. Steinhart[2], Z. Cohen[2], T. Delmonte[1], K. Kocher[1], K. Miller[1], S. Guschwan[1], R. McLeod[2], A. Cohen[4], D. Langelier[5], G. Lapointe[6], A. Griffiths[3], A. Bitton[4], G. Greenberg[2], E. Lander[1], K. Siminovitch[2], T. Hudson[4].* 1) Center for Genome Research,WI/MIT, Cambridge, MA; 2) Mount Sinai Hosp Lunenfeld Research Inst,Toronto, ONT; 3) Hosp for Sick Children, Toronto, ONT; 4) McGill University Health Center, Montréal, QC; 5) Centre Hospitalier de Sherbrooke, Sherbrooke, QC; 6) Centre Hospitalier de la Sagamie, Chicoutimi, QC.

We sought to design a systematic approach for LD mapping and apply it to localize a gene (*IBD5*) conferring susceptibility to CD. Using linkage analysis we had previously mapped the *IBD5* locus to a region spanning 18 cM of chromosome 5q31. Genotyping all known SSLP markers in this region in over 250 CD trios identified two adjacent markers with significant association to CD using TDT analysis. This was confirmed with additional markers designed from the available sequence. The boundaries of the implicated region were then defined using multi-locus methods to identify the underlying haplotypes conferring susceptibility. Single nucleotide polymorphism (SNP) discovery was performed by re-sequencing nearly 400 kb of the implicated region in 8 different DNA samples. Over 300 of these SNPs have been genotyped thus far in 139 CD trios. Fine structure haplotype analysis of this SNP data demonstrated that this region could be parsed into discrete blocks of LD (abstract: Daly, et al.). Moreover, we found that *IBD5* is contained within a common haplotype spanning about 250 kb that shows strong association with the disease ($p< 2x10^{-7}$). The precise disease-causing mutation cannot be readily identified from the genetic data alone, because strong LD across the region results in multiple SNPs with evidence consistent with that expected for the *IBD5* locus. The results have important implications for the genetic basis of IBD in particular and for LD mapping in general.

Program Nr: 248 from the 2001 ASHG Annual Meeting

Combined data analysis of four Finnish genome scans reveals a locus on chromosome 22 linked to dizygotic twinning. *M. Perola[1,2], J. Van Kirk[1], K. Sood[1], M. Öhman[2], T. Hiekkalinna[1], P. Pajukanta[1], M. Wessman[3,4], M. Koskenvuo[5], G. Montgomery[6], A. Palotie[1,4], J. Kaprio[7], L. Peltonen[1,2]*. 1) University of California, Los Angeles, Department of Human Genetics; 2) National Public Health Institute, Department of Molecular Medicine, Helsinki, Finland; 3) University of Helsinki, Department of Clinical Chemistry, Finland; 4) University of Helsinki, Department of Biosciences, Division of Genetics, Finland; 5) University of Turku, Department of Public Health, Finland; 6) Queensland Institute for Medical Research, Brisbane, Australia; 7) University of Helsinki, Department of Public Health and Department of Mental Health, National Public Health Institute, Helsinki Finland.

In recent years, numerous genome scans have been performed to identify loci predisposing to various traits. This data could be used in studies of common traits of biological significance, the re-analysis being justified by non-biased ascertainment of study individuals, if they have provided informed consent for such studies. We have recently reported the analysis of the genotypes collected in genome scans for common diseases to identify QTL of stature and BMI (Perola et al. AJHG in press). Here we report analysis of the families collected through a population-wide cohort of Finnish twins, consented for genetic analyses. We analyzed our families for twinning using the strategy applied by Busjahn et al. (NatGen 26:398-9), who recently reported a locus linked to dizygotic twinning in chromosome 3. The non-twin family members were included when possible as "non-affected". The study sample consisted of 140 families including 246 dizygotic twins and 97 parents or non-twin siblings. Genotypes were collected in four genome scans, with approximately 350 evenly spaced markers on 22 autosomes. The highest multipoint lod score (dominant model, 90% penetrance, no phenocopies) of 4.41 was found on chromosome 22 qter. No evidence of linkage was found on other chromosomes. Our data provide evidence for another locus for dizygotic twinning in Finnish study sample ascertained for late onset disease traits, possibly involved in the intrauterine survival of twins.

Program Nr: 249 from the 2001 ASHG Annual Meeting

A genome wide screen for loci involved in Specific Language Impairment (SLI). *D.F. Newbury[1], the SLI Consortium (SLIC)[1,2,3,4,5,6]*. 1) The Wellcome Trust Centre for Human Genetics, Univ. Oxford, UK; 2) Inst. Child Health, Guys Hospital, London, UK; 3) CLASP, Dev. Psych., Univ. Cambridge, UK; 4) Dept Exp. Psych., Univ. Oxford, UK; 5) Centre for Educational Needs, Univ. Manchester, UK; 6) Dept Child Health, Univ. Aberdeen, UK.

Approximately 4% of English speaking children are affected by Specific Language Impairment (SLI); a deficit in language abilities despite adequate opportunity and normal non-verbal intelligence. Family and twin studies have verified the importance of genetic factors in the development of SLI. However, like many behavioural traits, SLI is assumed to be complex in nature with several quantitative trait loci (QTLs) contributing to the genetic risk. In an attempt to map these loci the SLI Consortium (SLIC) has completed the first genome wide scan involving 210 sib pairs taken from 97 nuclear families, all with at least one child with language scores 1.5SD below that expected for their age. Linkage was assessed for three standardised language measures; The Clinical Evaluation of Language Fundamentals (CELF-R) battery was used to derive scores of expressive and receptive language abilities and a test of non-word repetition provided a marker of phonological short term memory. Families originated from two sources; A clinical study by Guys Hospital, and an epidemiological survey by the Cambridge Language and Speech Project (CLASP). Multipoint Haseman-Elston linkage analysis revealed two distinct loci with LOD scores above 3.0, both supported by variance component modelling. Separation of the data into the constituent groupings showed that the epidemiological and clinical samples contribute on equal terms to each of these QTLs thus providing an internal replication of linkage. This study implicates two novel locations in the search for susceptibility genes for language impairment. Neither of these overlap with SPCH1, the chromosome 7q locus previously identified in a unique family with a monogenic speech and language disorder. The refinement of the regions reported here may allow the identification of causal genetic factors in SLI and thus aid in the clarification of the aetiological mechanisms behind this disorder.

Program Nr: 250 from the 2001 ASHG Annual Meeting

Clinical delineation and localization to chromosome 9p13.3-p12 of a unique dominant disorder: Hereditary inclusion body myopathy, Paget disease of bone and frontotemporal dementia in four families. *V.E. Kimonis[1], M. Kovach[1], B. Waggoner[1], S.M. Leal[2], Z. Simmons[3], R. Khardori[4], M.P. Whyte[5], A. Pestronk[6].* 1) Div of Genet. & Metab., Dept. of Ped., Southern Illinois Univ. Sch. Med, Springfield, IL; 2) Rockefeller Univ., New York, NY; 3) Pennsylvania State Univ., Hershey, PA; 4) Dept. of Med., Southern Illinois Univ. Sch. Med., Springfield, IL; 5) Div of Bone and Mineral Dis, Washington Univ. Schl. of Med., St Louis, MO; 6) Dept. of Neurology, Washington Univ. Schl of Med., St Louis, MO.

The clinical, biochemical, radiological and pathological characteristics of 49 affected (23 M, 26 F) individuals from 4 unrelated families with this unique autosomal dominant disorder(MIM 605382) are reported. Of these individuals 44/49 (90 %) have myopathy, 21/49 (43 %) have PDB and 18/49 (37 %) frontotemporal dementia. Muscle histology reveals myopathic changes with blue rimmed vacuoles. Ultrastructural examination reveals filaments of phosphorylated tau, b-amyloid precursor protein epitopes and apolipoprotein E. PDB caused by overactive osteoclasts primarily involves the spine and hip, causes pain, elevated alk. phosphatase, and urine pyridinoline/ deoxypyridinoline and is responsive to bisphosphonates. Frontotemporal dementia occurs at a mean age of 54 y and contributes to the early demise from respiratory and cardiac failure secondary to the progressive muscle weakness. A genome-wide screen in the large Illinois family revealed linkage to chromosome 9 with a maximum LOD score of 3.64 with marker D9S301. Linkage analysis with a high density of chromosome 9 markers generated a maximum two-point LOD score of 7.62 for D9S1791, with a maximum multipoint LOD score of 12.24 between markers D9S304 and D9S1788. Subsequent identification and evaluation of three additional families demonstrating similar clinical features has allowed for localized to a 1.08-6.46 cM critical interval on 9p13.3-12 in the region of autosomal recessive IBM2. Identification of the putative gene is in progress and will no doubt provide insight into the common pathogenesis of this unusual combination of disorders.

Program Nr: 251 from the 2001 ASHG Annual Meeting

Gene-Gene interaction increases susceptibility to asthma: IL4Ra **and IL13 polymorphisms in an asthmatic Dutch population.** *T.D. Howard[1], G.H. Koppelman[2,3], J. Xu[1], S.L. Zheng[1], D.S. Postma[2], D.A. Meyers[1], E.R. Bleecker[1]*. 1) Center for Human Genomics, Wake Forest Univ Sch of Med, Winston-Salem, NC; 2) Department of Pulm, University Hospital, Groningen, the Netherlands; 3) Dept of Pulm, Rehab Beatrixoord, Haren, the Netherlands.

Asthma is a common respiratory disease characterized by variable airways obstruction caused by acute and chronic bronchial inflammation. Clinical findings in asthma include bronchial hyperresponsiveness and allergic responses, demonstrated by elevated total serum IgE levels and positive skin tests to common allergens. These closely associated phenotypes have been shown to have a strong genetic component. Binding of IL4 to the IL4 receptor (IL4R) induces the initial response for Th2 polarization. IL4 and IL13 are both produced by Th2 cells and are capable of inducing isotype class-switching of B-cells to produce IgE after allergen exposure. These cytokines also share a common receptor component, IL4Ra, which is a potential biological candidate gene for asthma and atopy. We have investigated five IL4Ra single-nucleotide polymorphisms in a well-characterized population of Dutch families ascertained through a proband with asthma. Using the probands and their spouses from this population in a case-control study design, we observed significant associations of atopy and asthma related phenotypes with several IL4Ra polymorphisms genotyped within the gene. The most significant association was observed with S478P, which was associated with high IgE levels ($p = 0.0007$). A significant gene-gene interaction was detected between the S478P variation in IL4Ra (significantly associated with high IgE levels) and the -1111 promoter variation in IL13 (significantly associated with bronchial hyperresponsiveness). Individuals with the risk genotype for both of these genes were at almost five times higher risk for the development of asthma compared to individuals with both non-risk genotypes ($p = 0.0004$). These data suggest that variations in IL4Ra contribute to elevated total serum IgE levels, and interaction between IL4Ra and IL13 markedly increases an individual's susceptibility to the development of asthma.

Program Nr: 252 from the 2001 ASHG Annual Meeting

Functional dissection of a human klotho allele that influences longevity. *D.E. Arking, H.C. Dietz.* HHMI & Inst Genet Med, Johns Hopkins SOM, Baltimore, MD.

Disruption of the mouse *Klotho* gene results in a syndrome resembling human aging, including atherosclerosis, emphysema, osteoporosis, and infertility. *Klotho* encodes a putative family 1 glycosidase that exhibits 20-40% sequence identity to b-glucosidases from bacteria and plants as well as mammalian lactase glycosylceramidase. Using a population-based association study, we previously demonstrated a role for *KLOTHO* in human aging. We identified an allele of *KLOTHO*, termed KL-VS, defined by the presence of six mutations in complete linkage disequilibrium, including two missense mutations, F354V and C372S. A homozygous disadvantage for KL-VS carriers was seen in three ethnically distinct populations ($p < 0.0023$). To assess the potential functional significance of the amino acid variations encoded by the KL-VS allele, we initially generated a Hidden Markov Model (HMM). The HMM predicts that the C372 residue is in the active site, sandwiched between the nucleophile and proton donor, and is therefore likely to directly influence the catalytic function and/or substrate binding/recognition properties of klotho. In contrast, F354 is likely to be on the surface of the protein, but may play an indirect role in catalysis. In a transient transfection assay, extracellular levels of klotho protein harboring V354 are reduced 6-fold, without a decrease in protein synthesis or stability, suggesting reduced secretion from the cell. Surprisingly, extracellular levels of klotho harboring S372 are increased 2.9-fold. The V354/S372 double-mutant exhibits an intermediate phenotype (1.6-fold increase), providing a rare example of intragenic complementation in *cis* by human SNPs. The remarkable evolutionary conservation of F354 among homologous proteins suggests that it is likely to be important to klotho function. Indeed, the substitution of valine for phenylalanine at the corresponding residue in the closest human klotho paralog with a known substrate, cBGL1, completely eliminates the ability to cleave p-nitrophenyl-b-D-glucoside. These data suggest that the KL-VS allele influences the trafficking and catalytic activity of klotho. Further insight regarding the pathogenesis of age-related phenotypes awaits identification of the precise substrate(s).

Program Nr: 253 from the 2001 ASHG Annual Meeting

Triallelic inheritance in Bardet-Biedl syndrome, a recessive, genetically heterogeneous, and pleiotropic disorder.

N. Katsanis[1], S.J. Ansley[1], J.L. Badano[1], E.R. Eichers[1], R.A. Lewis[1], B. Hoskins[2], P.J. Scambler[2], W.S. Davidson[3], P.L. Beales[2], J.R. Lupski[1]. 1) Baylor College of Medicine, Houston, TX, USA; 2) Institute of Child Health, UCL, London, UK; 3) Simon Frasier University, Vancouver, BC, Canada.

Bardet-Biedl syndrome (BBS) is a genetically heterogeneous disorder characterized by multiple clinical features that include pigmentary retinal dystrophy, polydactyly, obesity, developmental delay, and renal defects. Analysis of pedigree structures let historically to the hypothesis that this clinically symmetric disorder is inherited in an autosomal recessive fashion; subsequent positional cloning efforts identified the first two of at least seven *BBS* genes (*BBS2* and *BBS6*). We have screened our cohort of 163 BBS families for mutation in both *BBS2* and *BBS6* and, when possible by family size, have constructed haplotypes across all known *BBS* regions. We report the presence of three mutant alleles in affected individuals in each of four BBS pedigrees. Patients in three pedigrees have two mutations in *BBS2* and one mutation in *BBS6*, and the converse occurred in the fourth pedigree. In addition, we have identified a single *BBS2* mutation in four pedigrees that have been excluded genetically from both *BBS2* and *BBS6*. Finally, in two pedigrees segregating three *BBS* mutant alleles, we have identified unaffected individuals who carry two *BBS2* mutations but not a *BBS6* mutation. We therefore propose that BBS may not be a single-gene recessive disorder but a complex trait, possibly requiring the participation of multiple loci to manifest the phenotype. This model of disease transmission may be important in the study of genetic heterogeneity in recessive disorders and for modeling gene interactions in complex traits.

Program Nr: 254 from the 2001 ASHG Annual Meeting

Mutations of *CLCN2*, encoding a voltage-gated chloride channel, causing common forms of epilepsy. *K. Haug[1], A. Ramirez[1], T. Sander[2], M.I. Niemeyer[3], C. Kubisch[1], J. Rebstock[4], S. Horvath[5], K. Hallmann[1], J.S. Dullinger[1], B. Rau[1], F. Haverkamp[6], S. Beyenburg[4], H. Schulz[2], S.V. Sepulveda[3], D. Janz[2], C.E. Elger[4], P. Propping[1], L.P. Cid[3], A. Heils[1,4].*
1) Department of Human Genetics, University of Bonn, Germany; 2) Department of Neurology, University Hospital Charite, Berlin, Germany; 3) Centro de Estudios Cientficos (CECS), Valdivia, Chile; 4) Department of Epileptology, University of Bonn, Germany; 5) Departments of Human Genetics and Biostatistics, University of California, Los Angeles, USA; 6) Department of Pediatrics, University of Bonn, Germany.

Human epilepsy is a common neurological disorder, which affects about 2% of the population worldwide. Idiopathic generalized epilepsy (IGE) accounts for 40% of all epilepsies and comprises seven clinically defined syndromes, which are characterized by recurrent unprovoked generalized seizures without any detectable brain lesion. It is well established that the overlapping common IGE subtypes childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME) and generalized tonic clonic seizures (GTCS) on awakening are inherited as genetically complex traits. A recent genome-wide linkage analysis in complex inherited IGE identified a novel IGE susceptibility locus on chromosome 3q26. Using a positional candidate gene approach, we identified three different mutations in the voltage-gated chloride channel gene *CLCN2*, which co-segregate with the disease status in three IGE multiplex families. One of these mutations, a G to E exchange in the putative cytoplasmic carboxy-terminus, leads to a significantly slower current activation in transiently transfected HEK 293 cells. We furthermore detected a novel common coding polymorphism, which is associated with IGE as shown by the significant result of a family based association study in 115 parent/child trios (p=0.00015). Thus, *CLCN2* is the first gene, in which both rare mutations and a common sequence variation confer a range of varying susceptibility effects to genetically complex epilepsies.

Program Nr: 255 from the 2001 ASHG Annual Meeting

A polymorphism in the TCF7 locus is associated with type 1 diabetes in Caucasians. *J.A. Noble[1], A. White[1], D.B. Mirel[2], A.M. Valdes[3], R. Reynolds[2], G. Zangenberg[2], L. Lazzeroni[4], A. Grupe[5], G. Peltz[5], H.A. Erlich[1,2].* 1) Childrens Hosp Oakland Res Ins, Oakland, CA; 2) Roche Molecular Systems, Alameda, CA; 3) Incyte Genomics, Cambridge, UK; 4) Stanford University, Stanford, CA; 5) Roche Bioscience, Palo Alto, CA.

The transcription factor TCF-7, expressed in T cells, forms a complex with other proteins, including beta-catenin and activates several genes, including IL-12 receptor beta2 chain, characteristic of Th1 responses. Recently, we identified a novel, non-synonymous single-nucleotide polymorphism (SNP), with allele frequencies of 0.88 and 0.12 among Caucasians, in the human TCF-7 gene on chromosome 5q31. Based on recent mouse data, we hypothesized that the products of the two TCF-7 alleles might differ in their effects on Th1 and Th2 immune responses and that the two alleles might show differential associations with Th1- and Th2-mediated diseases. To address the latter hypothesis, we genotyped a large population of family-based samples from the Human Biological Data Interchange (HBDI) respository. These families were selected for the presence of type 1 diabetes, an autoimmune disease characterized by an Th1 type of autoreactive response to specific islet cell antigens. The collection included 283 families with unaffected parents and an affected sib pair. Disease association was evaluated by measuring the transmission of the TCF-7 alleles from heterozygous parents to affected offspring using the Transmission Disequilibrium Test (TDT). An increase in the transmission of the less frequent allele was observed. Stratification of the data by HLA class II genotype revealed that the increase in transmission could be seen primarily in those patients whose HLA genotype was not the highest-risk type 1 diabetes genotype (DR3/DR4-DQB1*0302). Moreover, the increase was seen in transmission from fathers ($p < 0.001$, RR= 1.64) and not from mothers. We examined whether the TCF-7 SNP might be causative or is merely a marker in linkage disequilibrium with a causative polymorphism both by mathematical modeling and by genotyping surrounding SNPs. Our data suggest that this TCF-7 polymorphism may itself represent a risk factor for type 1 diabetes.

Program Nr: 256 from the 2001 ASHG Annual Meeting

Application of kinetic PCR and molecular beacon technology to pooled sample and high-sensitivity analysis of the MTHFR gene. *M. Shi[1], J. Dagle[2], D. Caprau[2], J.C. Murray[2].* 1) Dept Biological Sci, Univ Iowa, Iowa City, IA; 2) Dept Pediatrics, Univ Iowa, IA.

The need to study thousands or tens of thousands of samples in an efficient manner from large populations with complex disorders can be effectively accomplished by the application and modification of new technologies. We have made use of real-time PCR to develop assays for the common C677T MTHFR thermolabile variant, which has already been studied widely in cardiovascular disease and birth defects. The application of a kinetic PCR approach has enabled us to carry out pooled sample analysis utilizing pools of approximately 100 individuals with success in pooled sample allele discrimination down to the two to three percent level. Pooled results are validated by individual genotyping assay and carried out in triplicate. DNA sample quality and quantification are important issues that were addressed in this pilot study. We have also developed a set of molecular beacon probes to analyze the same variant and demonstrated that the assays can be carried out reliably in 2.5 microliter volumes and with less than 100 picograms of DNA. This makes highly efficient use of expensive reagents and also provides opportunities to carry out thousands of genotypes even when samples have been obtained from highly restrictive sources, such as buccal swabs or newborn blood spots. The application of these technologies has been applied to several different populations with non-syndromic forms of cleft lip and palate with no significant associations demonstrated. These approaches are now being extended to a variety of additional genes expected to be in wide spread use for studies of common diseases and include NAT1, TGFA, and CYP3A.

Program Nr: 257 from the 2001 ASHG Annual Meeting

Estimating founder contributions in an isolated population. *D.K. Nolan[1], N. Cox[1,2,3], C. Ober[1,2]*. 1) Committee on Genetics, University of Chicago, Chicago, IL; 2) Department of Human Genetics, University of Chicago, Chicago, IL; 3) Department of Medicine, University of Chicago, Chicago, IL.

Founder populations have well known advantages for complex trait mapping. A reduced number of segregating alleles and increased linkage disequilibrium in recently founded populations make these groups ideal for identifying genes of modest effect. However, for most populations, the exact number of founders is unknown and must be estimated from genetic data or historical records. As part of our mapping studies of the Hutterites, we are examining founder relatedness and their contributions to the extant population. We have pedigree information to the early 1700's for all living Hutterites, a founder population of European origin. Based on pedigree data, 50 female founders contribute only 17 mitochondrial lineages to the >30,000 extant individuals. Of these 17 lineages, three account for ~60% of the female lineages. Similarly, of the 38 male founders, three Y-lineages account for ~60% of all male lineages. DNA samples were available for 14 of the 17 female lineages; two individuals from each were selected for mitochondrial HVR1 sequencing. This analysis revealed only 12 unique sequences, all belonging to the two most common European haplogroups, implying that at least two sets of female founders are related through a common female ancestor, with one set matching for HVR2 and sequencing of the other set underway. Kinship between founders could reduce genetic diversity by limiting the number of unique chromosomes from which it is derived. Moreover, there is evidence that these founders did not contribute equally to the gene pool and the census number of founders does not equal the effective number. When founders contribute unequally or are closely related, genetic diversity is further reduced. Such relatedness among the founders could affect estimates of IBD-sharing among living individuals and impact mapping studies. Similar studies of autosomal haplotypes with minimal recombination are underway. These studies suggest that the Hutterites have a more restricted gene pool than previously thought. Supported by HL56399 and HD21244.

Program Nr: 258 from the 2001 ASHG Annual Meeting

Mitochondrial DNA and Y-chromosome haplotypes of Gullah-speaking African Americans show closer genetic distance to Sierra Leoneans and lower Caucasian admixture than other New World African populations. *D.C. McLean, G.P. Page, W.T. Garvey.* Endocrinology, Medical University of South Carolina, Charleston, SC.

Gullah-speaking African Americans of the Sea Islands of South Carolina and Georgia (i.e., Gullahs) are reported to be genetically more homogeneous than African Americans from other regions and thus offer perhaps the best African American population for genetic epidemiology studies. Our hypothesis was that the Gullah are genetically closer to native Sierra Leoneans (Africans) than other New World African populations (represented by African Americans living in Charleston, S.C.; west coast African Americans; and Jamaicans) as measured by admixture metrics and genetic distances. We used mitochondrial DNA (mtDNA) and Y-chromosome polymorphisms to study maternal and paternal lineages, respectively. From initial mtDNA sequencing, we identified three novel, informative mtDNA restriction site polymorphisms and, together with four established mtDNA polymorphisms, constructed a seven position mtDNA haplotype for 1395 individuals. Y-chromosomes (n = 501) were haplotyped at four short tandem repeat loci plus the YAP insertion/deletion. Resulting highly reticulated networks for both mtDNA and Y-chromosome haplotypes show good distinction of Caucasian from African haplotypes. Caucasian admixture estimates in the New World African samples show higher paternal than maternal admixture. For both mtDNA and Y there is a sharp gradient of Caucasian admixture from a geographic core sub-sample of Gullah (mt: 0.0605 Y: 0.1027), through the larger Gullah community in the rural S.C. Lowcountry (mt: 0.0648 Y: 0.1704), to urban African Americans living in Charleston (mt: 0.1004 Y: 0.2661). Genetic distances show that the Gullah are genetically closer to Sierra Leoneans than are other New World African populations included in the study. Principal coordinates analyses of genetic distances show that the first principal coordinate explains the majority of Caucasian to African distance while the second explains much of the distances among the included African-origin samples. These studies affirm the uniqueness and appropriateness of using the Gullah community for additional genetic epidemiology studies.

Program Nr: 259 from the 2001 ASHG Annual Meeting

Defining and Typing Ethnic Difference Markers in Admixed Populations for Estimating MALD Analytic Power.

H. Collins-Schramm[1], H. Li[1], J. Weber[2], R. Cooper[3], C. Phillips[1], D. Operario[1], J. Lee[1], M.F. Seldin[1]. 1) Rowe Program in Human Genetics, UC Davis, Davis, CA 95616; 2) Marshfield Medical Research Foundation, Marshfield, WI 54449; 3) Preventive Medicine and Epidemiology, Loyola Univ, Maywood, IL 60153.

Mapping by Admixture Linkage Disequilibrium is a potentially powerful technique for mapping disease susceptibility genes in complex genetic disease. The method uses the increased levels of linkage disequilibrium in admixed populations such as today's Mexican Americans (MA) or African Americans (AA). MALD requires a genome wide set of markers with large allele frequency differences (d) between the parental populations, such as between European American (EA) and African (A) for use in AA MALD studies or between EA and Amerindian (AI) for use in MA MALD studies. By screening over 800 microsatellite and insertion/deletion markers, we have identified >200 with ds likely to be useful for MALD in MA and AA populations. Markers in this subset have small ds between subgroups of a population (e.g. between Pima and Yavapai Indians) and modeling suggests that modern ethnic populations are reasonable representatives of contributing parental population subgroups (e.g. Zimbabwe Africans for A, Pima Indians for AI and EA for European derived populations). We have used these markers to genotype approximately 200 individuals from each parental ethnicity and 400 individuals from each admixed ethnicity for two chromosomal regions. Statistical multipoint analysis using the Structure program and haplotype analysis using the Arlequin program were used to examine the genetic composition of today's MA and AA populations over these regions, and simulations to derive the power of MALD under varying conditions were performed. Results support that both AA and MA populations are likely to be useful for MALD analysis and demonstrate that MALD can achieve promising power under conditions such as those expected in complex genetic disease (e.g. 0.8 for a sample size of 600 case and control AAs at a relative risk of 2.0). In addition, results show that individual typing of the parental populations may not necessarily be a requirement for MALD analysis.

Program Nr: 260 from the 2001 ASHG Annual Meeting

A theoretical framework for Mapping by Admixture Linkage Disequilibrium (MALD) with application to Lipoprotein(a) [Lp(a)] in African-Americans (AAs). *J. Chretien[1], J. Coresh[1], N.E. Fink[1], M.J. Klag[1], S.M. Marcovina[2], M.W. Smith[3]*. 1) Johns Hopkins University, Baltimore, MD; 2) University of Washington, Seattle; 3) National Cancer Institute at Frederick, MD.

The short range of linkage disequilibrium (LD) in human populations limits the feasibility of genome-wide marker association studies. In an admixed population, initial two-locus disequilibrium is proportional to the product of allele frequency differences (deltas) between parent populations. Sufficient LD may remain in recently admixed populations to map genes which underlie ethnic differences in phenotype distributions. We developed the Ethnicity Score (ES), a weighted sum of deltas for an individuals two marker alleles; the weight is the inverse of the allele frequency in the admixed population. We show that under standard admixture and inheritance models, the mean of a quantitative phenotype is a linear function of the marker ES. Linear regression of the phenotype on ES allows for testing whether the marker is linked to a locus which explains part of the phenotype distribution difference between parent populations. We derive expressions for variance and power. Generalized Linear Models allow for application to binary phenotypes. We apply the method to plasma level of the atherogenic particle Lp(a). Lp(a) level is higher in Africans and AAs than European-Americans (EAs) and in persons with kidney failure. The apo(a) locus has been proposed as the cause of the ethnic difference but no direct test of this hypothesis exists. The study population is 200 AA and 450 EA kidney failure patients in the CHOICE Study. Lp(a) level was measured at baseline and 14 STRs were typed within 15 cM of the apo(a) gene. Median Lp(a) in AAs and EAs was 88.5 and 36.3 nmol/L, respectively. We found significant positive associations between ES and ln[Lp(a)] at 0 cM (promoter PNRP, P=.001; D6S1579, P=.002), -5 cM (D6S437, P=.015), and 13 cM (D6S264, P=.048) from apo(a) in AAs but for no STRs in EAs. These results suggest that apo(a) locus variation contributes to higher Lp(a) in AAs, and that the ES method is an effective implementation of MALD which may help map other phenotypes which differ between parental populations.

Program Nr: 261 from the 2001 ASHG Annual Meeting

Long-range Linkage Disequilibrium in the African American Genome. *S.K. Iyengar[1], B. Rybicki[2], R. Liptak[1], T. Harris[1], M. Maliarik[2], M. Iannuzzi[2], R.E. Elston[1]*. 1) Case Western Reserve Univ, Cleveland, OH; 2) Henry Ford Health System, Detroit, MI.

Quantification of linkage disequilibrium (LD) in the African-American (AA) genome has relevance to mapping genes for multifactorial diseases. Although LD has been extensively explored in Caucasian (CA) populations, a comprehensive study of LD has not been conducted in the AA genome. We investigated if population-specific variables, such as differences in allele frequencies and admixture, influence the extent of LD in the AA genome. Polymorphic microsatellites (N=99), located 2-3 cM apart, in gene-rich regions spanning approximately 270 cM on chromosomes 1, 3, 11, 12, 13, 14, 15, 16, 17, 20 and 22 were genotyped. Maximum-likelihood haplotype frequencies were computed using the E-M algorithm for 200 unrelated African Americans that participated in a sarcoidosis family study. Statistically significant LD was estimated empirically by comparing the likelihood of the estimated haplotype frequencies with the likelihood of haplotypes simulated under the assumption of linkage equilibrium. Results are described for 8 of the 11 chromosomal regions, comprising 88 markers. Strong evidence for LD on chromosome 11 in the AA genome was observed, where 3 pairs of consecutive markers approximately 2.9 to 3.2 cM apart exhibit significant ($p<0.05$) pairwise LD. Long range LD was also observed among chromosome 11 markers 7.56, 10.43, and 13.36 cM apart. The evidence for long range LD on chromosomes 12, 14 and 22 was weaker, but several significant pairwise results were observed at shorter distances of 2-5 cM. Additional LD was found between non-adjacent loci ranging from 10 to 19 cM apart. These results confirm the anticipated result that LD in the AA genome extends further than in the CA genome. Selection of subjects from the sarcoidosis family study may influence the extent of LD somewhat, but we anticipate that the majority of long range LD we observe will be due to recent admixture. Future analyses will correlate measures of LD with genetic distance between locus pairs, relative position of locus pairs on the chromosome, gene density and genetic distance from the Caucasian CEPH population.

Program Nr: 262 from the 2001 ASHG Annual Meeting

Extent and Pattern of Gene Diversity at Microsatellite Loci: Implications for Disease-Gene Association Studies.

R. Chakraborty[1], L. Jin[1], R. Deka[2], M. Kimmel[3]. 1) Human Genetics Ctr, Univ Texas Health Sci Ctr, Houston, TX; 2) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 3) Dept Statistics, Rice Univ, Houston, TX.

Population-based disease-gene association studies conducted with either the candidate gene or genome-scan approach require adjustments for possible population substructure effects that might influence the association results. Since at present the dense map of the human genome is based on microsatellite loci, and the anticipation is that with single nucleotide polymorphic sites, the density will increase, this investigation addresses the theoretical issues of the extent and pattern of gene diversity at microsatellite loci. We show that gene diversity at microsatellites can be studied by decomposing gene diversity (expected heterozygosity) or allele size variance. The coefficient of gene differentiation based on the decomposition of allele size variance has an expectation larger than the one based of heterozygosity. However, the variance-based measure is also accompanied with a larger sampling variance, yielding a lower power of detection of population substructure through the variance-based approach. Both measures of gene diversity, however, monotonically decrease with increasing mutation rates. Thus, at a genome level, loci located at regions of higher mutation rates should have a lesser impact of population substructure effects on genetic variation. The above pattern of dependence of the coefficient of gene diversity on mutation rate is also valid for the finite allele mutation model, and hence, it is expected that SNP-based estimates of coefficient of gene diversity may be larger than the one based on microsatellites, since mutation rates at SNP sites are smaller than that at microsatellite loci. In other words, in SNP-based disease-gene association studies a greater care of adjustments for population substructure effects might be needed.
(Research supported by NIH grants GM 41399, GM 45816, and GM 58545).

Program Nr: 263 from the 2001 ASHG Annual Meeting

Extensions to the Combinatorial Partitioning Method. *B.J. Renner[1], S.L.R. Kardia[2], R.E. Ferrell[3], C.F. Sing[1]*. 1) Dept Human Genetics, Univ Michigan, Ann Arbor, MI; 2) Dept Epidemiology, Univ Michigan, Ann Arbor, MI; 3) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

Genome technologies are making possible rapid measurement of thousands of SNP genotypes on large samples. Analytical methods that fit traditional genetic models to such data have limited utility for modeling the complexity of genotype-phenotype relationships. The Combinatorial Partitioning Method (CPM), which does not require an a priori genetic model, is a computational strategy for identifying subsets of sites and their multi-site genotypic partitions that predict interindividual trait variation. The CPM method has been extended to evaluate multilocus genotypes defined by three or more loci. The extended method was applied to variation in plasma triglyceride levels collected on 188 males, ages 20-60 yr, ascertained without regard to health status, from Rochester, Minnesota, and typed on 18 diallelic loci in six candidate CHD susceptibility gene regions. Previous analysis identified two markers from the LDLR gene and APOAI/CIII/AIV gene cluster that partition the 2-locus genotypes into three classes that predict 9.3% of the variation in triglyceride levels. Using the extended method, we find the addition of a marker in the Paraoxonase-3 (PON1) gene increases the predicted variation to 20.9% ($p < 0.05$). The increase in prediction obtained by addition of this third locus is an illustration of Simpsons Paradox and emphasizes the context dependent nature of genotype-phenotype relationships.

Program Nr: 264 from the 2001 ASHG Annual Meeting

Evidence for Positive Selection Acting at the Human Dopamine Receptor D4 Gene Locus. *R.K. Moyzis[1], Y.C. Ding[1,2], H.C. Chi[1], D.L. Grady[1], J.R. Kidd[3], A. Morishima[3], K.K. Kidd[3], P. Flodman[1], M.A. Spence[1], S. Schuck[1], J.M. Swanson[1], Y.P. Zhang[2]*. 1) Univ California, Irvine, CA; 2) Kunming Institute of Zoology, Kunming, China; 3) Yale University, New Haven, CT.

Associations have been reported of the 7-repeat (7R) allele of the human dopamine receptor D4 (DRD4) gene, located near the telomere of chromosome 11p, with both attention deficit/hyperactivity disorder(ADHD) and the personality trait of novelty seeking. This polymorphism occurs in a 48-bp tandem repeat in the coding region of DRD4. While the most common allele of DRD4 contains four repeats at this position (4R), and represents 65 percent of examined alleles, rarer variants containing from two (2R) to eleven (11R) repeat copies are also found. By PCR-based DNA resequencing of 600 DRD4 alleles, representing a worldwide population sample, numerous single nucleotide polymorphisms (cSNPs) in the 48-bp repeat were found. DNA sequence variability is lowest for the most common 2R, 4R, and 7R alleles. Fifty-six different DRD4 exon 3 haplotypes were found, most at low (<1%) frequency. Rare 2R- through 6R-alleles can be explained, however, by simple one-step recombination/mutation events between the most common alleles. In contrast, the formation of the observed 7R and higher-alleles cannot be explained in this manner. The generation of a 7R-allele from the most prevalent 4R-allele would require at least one recombination and multiple (>6) mutations and/or gene conversions. No putative intermediate alleles of DRD4-7R were observed in this worldwide sample, including samples obtained from Africa, thought to contain populations with the greatest genetic diversity and age. Interestingly, strong linkage disequilibrium exists between the 7R-allele and surrounding DRD4 polymorphisms. Calculations of allele age based on intra-allelic variability indicate that the 7R-allele, unlike the common 4R-allele, is at least 5-10 fold younger than suggested by calculations based on population frequency. Thus, it appears that the DRD4 7R-allele originated as a rare mutational event that never the less increased to high frequencies in some human populations, likely through direct positive selection.

Program Nr: 265 from the 2001 ASHG Annual Meeting

Variance-Component Linkage Methods for Non-Normally Distributed Trait Data. *M.P. Epstein, X. Lin, M. Boehnke.* Department of Biostatistics, University of Michigan, Ann Arbor, MI.

Variance-component methods of quantitative trait linkage analysis (Amos 1994; Almasy and Blangero 1998) have many attractive features that other quantitative trait linkage methods lack. Variance-component procedures require few assumptions and can accommodate many genetic phenomena including multiple gene and environmental effects and their interactions. Variance-component approaches also tend to have greater linkage power than relative-pair-based techniques, in part because variance-component methods analyze all individuals in a family simultaneously.

However, standard variance-component methods assume that the analyzed quantitative trait data in a family follow a multivariate normal distribution. If this assumption is violated, variance-component methods may yield biased results. This limits the application of these methods. We have chosen to extend the variance-component approach of linkage analysis to accommodate dichotomous, polychotomous, and truncated quantitative data. We still use the general variance-component modeling framework, but apply generalized linear mixed models (Breslow and Clayton 1993) to account for the non-normal nature of the data.

To illustrate our approach, we apply a logistic-mixed model to simulated binary disease trait data assuming a complex disease. This model analyzes all individuals (both affected and unaffected) simultaneously within a family. We will present power and type I error results assuming a variety of complex disease models. We will also discuss the effect of ascertainment bias and discuss methods for ascertainment adjustment. Preliminary simulation results suggest our proposed logistic-mixed model performs well in terms of size and power. As an example, we simulated a disease with a population prevalence of 1% and a sibling recurrence risk of 9. We then analyzed samples of 400 sibships each of size 3 with at least one affected sib per family. Our method yielded LOD scores >3 85% of the time when the gene of interest accounted for 75% of the total genetic variance.

Program Nr: 266 from the 2001 ASHG Annual Meeting

A new computational approach for rapid multipoint linkage analysis of qualitative and quantitative traits in large, complex pedigrees, and its implementation in GENEHUNTER. *K. Markianos[1], A. Katz[1], L. Kruglyak[1,2]*. 1) Fred Hutchinson Cancer Res Ctr, Seattle, WA; 2) Howard Hughes Medical Institute.

We present a new approach for rapid multipoint linkage analysis that takes into account both genotyping information and symmetries generated by missing ancestors to reduce the scale of multipoint inheritance calculations in large, complex pedigrees. At every genetic marker we use:
(1) Classification of meioses into those whose outcome is exactly determined from observed genotypes and those whose outcome is ambiguous. For example, a single meiosis with a known outcome reduces the number of inheritance patterns with non-zero inheritance probability at that marker by half.
(2) Classification of inheritance patterns into equivalence classes generated by observed genotypes (e.g. meioses that belong to a homozygous parent are equally probable) or by information lost due to ungenotyped ancestors.
We apply a series of transformations that allow us to factorize the computation for evaluation of single-point and multi-point inheritance probabilities. In typical pedigrees, use of the first set of methods alone (exactly broken symmetries) reduces the computational burden by a factor of 10-1000. The complementary set of methods (equivalence classes) introduces similar efficiency gains. We note that the first set of methods is most relevant with the present generation of highly polymorphic genetic markers (STRPs), while the second set of methods is most relevant for the next generation of highly abundant, but less informative genetic markers (SNPs).
The first set of methods has been implemented in the genetic analysis program GENEHUNTER. We are in the process of implementing the second set of methods, along with a series of practical improvements, including efficient calculation of parametric LOD scores in very large pedigrees, removal of limitations on the maximum number of meioses we can consider, and exact haplotyping. We comment on the influence of pedigree structure and genotyping strategies on the efficiency of the approach.

Program Nr: 267 from the 2001 ASHG Annual Meeting

Muscular dystrophy and neuronal migration disorder caused by mutations in a novel glycosyltransferase. *K. Kobayashi[1], A. Yoshida[2], H. Manya[3], M. Mamoru[4], T. Inazu[4], H. Mitsuhashi[2], H. Topaloglu[5], M. Takeuchi[2], T. Endo[3], T. Toda[1]*. 1) Division of Functional Genomics, Department of Post-Genomics and Diseases, Course of Advanced Medicine, Osaka University Graduate School of Medicine, Osaka, Japan; 2) Central Laboratories for Key Technology, Kirin Brewery Co., Ltd., Yokohama, Japan; 3) Department of Glycobiology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan; 4) Research Department, The Noguchi Institute, Tokyo, Japan; 5) Department of Paediatric Neurology, Hacettepe Children's Hospital, Ankara, Turkey.

Muscle-eye-brain disease (MEB) is an autosomal recessive disorder characterized by congenital muscular dystrophy, ocular abnormalities, and lissencephaly. Mammalian *O*-Mannosyl glycosylation is a novel protein modification observed in a limited number of glycoproteins of brain, nerve, and skeletal muscle. Here we isolated a human cDNA for *O*-linked mannose b1,2-*N*-acetylglucosaminyltransferase (OMGnT), which is a novel enzyme for mammalian type *O*-mannosyl glycan synthesis. The cDNA encoded a putative type II membrane protein of 660 amino acids having 23.2% sequence identity to human a-3-D-mannoside b-1,2-*N*-acetylglucosaminyltransferase I (GnT-I). By transfecting this cDNA into HEK293T cells, OMGnT activity increased approximately 100-fold. However, OMGnT did not show any detectable GnT-I activity. Northern blot analysis revealed that OMGnT is constitutively expressed in all human tissues tested. These results clearly demonstrate that OMGnT is a new b1,2-*N*-acetylglucosaminyltransferase functionally different from GnT-I. Moreover, we identified two independent mutations of this gene in two patients with MEB, indicating that the OMGnT gene is responsible for MEB. These findings would suggest a novel pathomechanism, glycosylation, on muscular dystrophy as well as neuronal migration disorder.

Program Nr: 268 from the 2001 ASHG Annual Meeting

A novel glycosyltransferase is mutated in a form of congenital muscular dystrophy with secondary laminin a**2 deficiency and abnormal glycosylation of** a**-dystroglycan.** *M. Brockington[1], D.J. Blake[2], P. Prandini[1], S.C. Brown[1], S. Torelli[1,3], M.A. Benson[2], C.P. Ponting[2], B. Estournet[4], N. Romero[5], T. Voit[6], C.A. Sewry[7], P. Guicheney[5], F. Muntoni[1]*. 1) The Dubowitz Neuromuscular Centre, ICSM,Hammersmith Hospital, London, UK; 2) Department of Human Anatomy and Genetics, University of Oxford, UK; 3) Department of Cytomorphology, University of Cagliari, Italy; 4) Hpital Raymond Poincar, Garches, France; 5) Inserm U 523, Pitie-Salpetriere, Paris, France; 6) Department of Paediatrics, University of Essen, Germany; 7) 7Department of Histopathology, Robert Jones & Agnes Hunt Orthopaedic Hospital, Oswestry, UK.

The congenital muscular dystrophies (CMD) are a heterogeneous group of autosomal recessive disorders, presenting in infancy with muscle weakness, contractures and dystrophic changes on skeletal muscle biopsy. Brain involvement is also present in several CMD syndromes. Approximately 40% of patients have a primary deficiency of laminin a2 due to mutations in the *LAMA2* gene. A secondary deficiency of laminin a2 is apparent in some CMD syndromes.

Here we identify a gene encoding a novel putative glycosyltransferase. We report the genomic organization of this locus and its pattern of tissue expression. Mutations in this gene have been identified in 7 families with a severe form of CMD that does not involve the brain. Patients have markedly elevated serum creatine kinase and a secondary deficiency of laminin a2. We observed a marked decrease of muscle a-dystroglycan immunostaining and a significant drop in its molecular weight on Western blot. These observations are interpreted to be the result of altered glycosylation of a-dystroglycan. This is likely to be integral to the pathology seen in these patients.

Program Nr: 269 from the 2001 ASHG Annual Meeting

A human homologue of yeast pre mRNA-splicing gene, *PRP31*, underlies autosomal dominant retinitis pigmentosa on chromosome 19q13.4 (*RP11*). *E.N. Vithana[1], L. Abu-Safieh[1], M.J. Allen[2], A. Carey[2], M. Papaioannou[1], C. Chakarova[1], M. Al-Maghtheh[1], N.D. Ebenezer[1], C. Willis[3], A.T Moore[1,3], A.C. Bird[3], D.M. Hunt[1], S.S. Bhattacharya[1]*. 1) Department of Molecular Genetics, Institute of Ophthalmology, University College London, London, United Kingdom; 2) Oxagen Limited, 91 Milton Park, Abingdon, Oxon, OX14 4RY, United Kingdom; 3) Moorfields Eye Hospital, City Road, London, United Kingdom.

Autosomal dominant Retinitis Pigmentosa (adRP) is an inherited progressive retinal degeneration characterised by night blindness and constricted visual fields. adRP is genetically heterogeneous, whilst mutations have only been found in five genes to date genetic linkage studies have implicated at least six further loci. We now report the cloning of the adRP gene on chromosome 19q13.4 (*RP11*; MIM 600138). The use of positional candidate strategy supported by bioinformatics led to the identification of the *RP11* gene, which is homologous to Saccharomyces cerevisiae pre-mRNA splicing gene *PRP31*, comprising 14 exons and encoding a protein of 499 amino acids. RT PCR analysis also indicated *PRPF31* to be widely expressed. Upon sequence analysis we identified mutations in four *RP11* linked families and three sporadic RP cases. Mutations include missense alterations deletions and insertions. The identification of mutations in a pre-mRNA splicing gene implicates defects in the splicing process as a novel mechanism of photoreceptor degeneration.

Program Nr: 270 from the 2001 ASHG Annual Meeting

Mutated POLG associated with progressive external ophtalmoplegia characterised by multiple mtDNA deletions.

G. Van Goethem[1,2], B. Dermaut[1], A. Löfgren[1], J-J. Martin[2], C. Van Broeckhoven[1]. 1) Department of Molecular Genetics, Flanders Interuniversity Institute for Biotechnology (VIB), Born-Bunge Foundation (BBS), University of Antwerp (UIA); 2) Division of Neurology, University Hospital of Antwerp (UZA), Antwerpen, Belgium.

Autosomal dominant and recessive progressive external ophtalmoplegias (adPEO and arPEO) are rare human diseases, characterized by accumulation of large-scale mitochondrial DNA (mtDNA) deletions. We mapped a novel locus for adPEO at 15q22-q26 in a Belgian pedigree and identified a missense mutation (Y955C) in the polymerase motif B of the mtDNA polymerase (POLG). Y955 is highly conserved and is critical for mtDNA replication. In two other nuclear Belgian families with PEO and multiple mtDNA deletions we identified 3 different POLG missense mutations compatible with arPEO. These mutations are in evolutionary conserved POLG residues. Our finding of POLG mutations provides significant evidence for a crucial role for POLG in the pathogenesis of mitochondrial disorders due to accumulation of deleted mtDNA molecules.

Program Nr: 271 from the 2001 ASHG Annual Meeting

Homozygous mutations in PHOX2A result in Congenital Fibrosis of the Extraocular Muscles type 2 (CFEOM2).

E.C. Engle[1], M. Nakano[1], J. Fain[1], C.J. Selleck[2], A.H. Awad[2], P.B. Mullaney[2], J. Zwann[3], E.C. Sener[4]. 1) Dept Genetics & Neurology, Children's Hosp, Harvard Medical School, Boston, MA; 2) King Khaled Eye Specialist Hospital, Riyadh, Kingdom of Saudi Arabia; 3) Ophthalmology, University of Texas Health Science Center, San Antonio, TX; 4) Ophthalmology, Hacettepe University, Ankara, Turkey.

Isolated strabismus affects 1-5% of the general population. Most forms of strabismus are multifactorial in origin and, while there is likely an inherited component, the genetics of these disorders remain unelucidated. The congenital fibrosis syndromes represent a subset of monogenic isolated strabismic disorders that are characterized by restrictive ophthalmoplegia, and include congenital fibrosis of the extraocular muscles (CFEOM) and Duane syndrome. Neuropathologic studies suggest that these disorders result from maldevelopment of the oculomotor (nIII), trochlear (nIV), and abducens (nVI) cranial nerve nuclei. To date, five congenital fibrosis syndrome genetic loci have been mapped (CFEOM1-3, DURS1-2), but no genes have been identified. We previously mapped CFEOM2, characterized by recessive exotropic ophthalmoplegia and ptosis, to chromosome 11q13 in three consanguineous Saudi Arabian families, two of whom share a disease-associated haplotype. We have subsequently identified a consanguineous Turkish CFEOM2 pedigree with a third recessive haplotype. We undertook a positional cloning effort to identify the CFEOM2 gene and now report three mutations in PHOX2A (ARIX), a gene encoding a homeodomain transcription factor protein previously shown to be required for nIII/nIV development. Two mutations are predicted to disrupt splicing, while the third alters an amino acid within the conserved brachyury-like domain. These findings confirm the hypothesis that CFEOM2 results from the abnormal development of nIII/nIV and emphasize the critical role for PHOX2A in the development of these midbrain motor nuclei in addition to its established function in the development of the adrenergic nervous system.

Program Nr: 272 from the 2001 ASHG Annual Meeting

Mutations in the retinitis pigmentosa GTPase regulator interacting protein (RPGRIP1) gene are responsible for Leber congenital amaurosis. *S. GERBER[1], I. PERRAULT[1], S. HANEIN[1], F. BARBET[1], D. DUCROQ[1], I. GHAZI[2], J.-L. DUFIER[2], A. MUNNICH[1], J. KAPLAN[1], J.-M. ROZET[1]*. 1) Laboratoire de Recherches sur les Handicaps Genetiques de l'Enfant, INSERM U393, Hopital des Enfants Malades, PARIS, FRANCE; 2) Service d'Ophtalmologie, Hopital Necker, Paris, France.

Leber congenital amaurosis (LCA) is a genetically heterogeneous autosomal recessive condition responsible for congenital blindness or greatly impaired vision since birth. So far, four LCA genes have been identified. Two additional loci have been mapped by linkage analyses, each in a single large family. A genome-wide screen for homozygosity was conducted in seven consanguineous families unlinked to any of the 6 LCA loci. Evidence for homozygosity for chromosome 14q11 markers was found in two of the seven families. A retinal-specific candidate gene the retinitis pigmentosa GTPase regulator interacting protein (RPGRIP1), was known to map to this region. We screened the 14 known coding exons of this gene in the two families consistent with linkage to chromosome 14q11. This screening allowed the identification of a homozygous missense mutation in one of the two families. However, no mutation was found in the second family. Subsequently, we determined the complete exon-intron structure of the RPGRIP1 gene by comparison with the mouse and bovine structures, and identified 9 novel exons and their corresponding exon-intron boundaries. Subsequently, we identified a homozygous null mutation in one additional exon, in the second consanguineous family linked to 14q11. Further screening of LCA patients unlinked to any of the four known LCA genes (n = 86) identified seven additional mutations in six of them. In total, eight distinct mutations (5/8 truncating) in 8/93 patients were found. So far this gene accounts for 8/142 LCA cases in our series (5.6 %).

Program Nr: 273 from the 2001 ASHG Annual Meeting

A mutation in *MYO6*, the human homologue of the gene causing deafness in Snell's waltzer mice, is associated with hearing loss. *K.B. Avraham[1], N. Ahituv[1], S. Melchionda[2], L. Bisceglia[2], T. Sobe[1], F. Glaser[3], R. Rabionet[4], M.L. Arbones[4], A. Notarangelo[2], E. Di Iorio[2], L. Zelante[2], X. Estivill[4], P. Gasparini[2].* 1) Department of Human Genetics & Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv, 69978, Israel; 2) Servizio Genetica Medica, IRCCS-Ospedale CSS, I-71013 San Giovanni Rotondo, Italy; 3) Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel; 4) Medical and Molecular Genetics Center IRO, Hospital Duran i Reynals, Barcelona, Catalonia, Spain.

Mutations in unconventional myosins have been identified in syndromic and nonsyndromic deafness in humans and mice. Unconventional myosins comprise a group of 16 nonfilament-forming myosins, defined by a conserved motor domain and different tail domains. An intragenic deletion in the unconventional myosin, myosin VI, was found to be the cause of deafness and vestibular dysfunction in Snell's waltzer mice.

The human homologue, *MYO6*, maps to chromosome 6q13. A genome wide scan of an Italian family with autosomal dominant progressive hearing loss localized the mutant locus to an 11 cM region on chromosome 6. The human *MYO6* gene maps within the candidate region and was thus an excellent candidate based on its chromosomal location, cochlear expression and function. Mutation analysis of the exons and exon-intron junctions revealed a G®A missense mutation in the coding region of this gene, which replaces a cysteine with a tyrosine at residue 442 of the protein (C442Y) (Genbank U90236). The mutation segregates with all affected individuals in the Italian family and was not identified in 200 control individuals.

The C442Y mutation affects a cysteine residue in the motor domain that is conserved across other myosin VI species. A 3D model of human myosin VI (based on the myosin II structure) enables us to predict the mechanism of this mutation. In order to further assess this mutation, transgenic mice expressing this mutation were generated. Preliminary results are promising, showing a progressive hearing loss in several founder mice.

Program Nr: 274 from the 2001 ASHG Annual Meeting

Mutations in the Wolfram syndrome 1 gene (WFS1) are a common cause of low frequency sensorineural hearing loss. *I.N. Bespalova[1], G. Van Camp[2], S. Bom[3], D.J. Brown[4], K. Cryns[2], A.T. DeWan[5], A.E. Erson[6], K. Flothmann[2], H. Kunst[3], P. Kurnool[4], T.A. Sivakumaran[4], C.W.R.J. Cremers[3], S.M. Leal[5], M. Burmeister[1,6,7], M.M. Lesperance[4].* 1) Mental Health Research Inst, Univ Michigan, Ann Arbor, MI; 2) Dept Medical Genetics, Univ Antwerp, Belgium; 3) ENT Dept, Univ Nijmegen, The Netherlands; 4) Dept Otolaryngology-Head and Neck Surgery, Univ Michigan, Ann Arbor, MI; 5) Lab Statistical Genetics, The Rockefeller University, New York, NY; 6) Dept Human Genetics, Univ Michigan, Ann Arbor, MI; 7) Dept Psychiatry, Univ Michigan, Ann Arbor, MI.

Nonsyndromic sensorineural hearing loss is overall a common disorder which is genetically very heterogeneous with over 60 loci mapped. In contrast, sensorineural hearing loss specifically affecting the low frequencies (below 2000 Hz) is an unusual type of hearing loss which is often asymptomatic and less likely to be diagnosed. We have previously reported two families with autosomal dominant LFSNHL linked to 4p16 (DFNA6/14) (Lesperance 1995, Van Camp 1999). LFSNHL appears to be genetically nearly homogeneous, as only one LFSNHL family is known to map to a different chromosome (DFNA1) (Lynch et al. 1997). The DFNA6/14 critical region includes WFS1, the gene responsible for the autosomal recessive Wolfram syndrome, characterized by diabetes mellitus, diabetes insipidus, optic atrophy, and deafness (Strom 1998). Herein we report 6 different heterozygous missense mutations (G674E, T699M, A716T, V779M, L829P, G831D) in WFS1. Mutations in WFS1 were identified in all families with LFSNHL tested. None of the mutations were found in at least 180 control chromosomes with the exception of V779M, identified in 1/180 controls of unknown hearing status (Collins et al. 1998). This frequency is consistent with the prevalence of heterozygous carriers of Wolfram syndrome estimated at 1/100 in some populations (Barrett 1995). An increased risk of sensorineural hearing loss has been reported in heterozygous carriers in families with Wolfram syndrome (Ohata 1998). We therefore conclude that mutations in WFS1 are a common cause of LFSNHL.

Program Nr: 275 from the 2001 ASHG Annual Meeting

Mutations in Kir2.1 cause the developmental and episodic electrical phenotypes of Andersen's Syndrome. *L.J. Ptacek[1,2], A. Soemori[2], M. Donaldson[2], N. Plaster[2], S. Bendahhou[2], M. Tristani-Firouzi[3], R. Tawil[4], Y.-H. Fu[5]*. 1) HHMI, EIHG Bldg 533, Rm 4420, Univ Utah, SLC, UT; 2) Departments of Neurology and Human Genetics, Univ Utah, SLC, UT; 3) Department of Pediatrics, Univ Utah, SLC, UT; 4) Department of Neurology, University of Rochester; 5) Department of Neurobiology and Anatomy, Univ Utah, SLC, UT.

Andersen's syndrome (AS) is characterized by periodic paralysis, cardiac arrhythmias, and dysmorphic features. The dysmorphic features include short stature, scoliosis, clinodactyly, hypertelorism, small or prominent ears that are low set or slanted, cleft palate, micrognathia, and broad forehead. In AS families, expression of the characteristic traits is highly variable. AS occurs either sporadically or as an autosomal dominant trait. We have mapped an Andersen's locus to chromosome 17q23 (maximum LOD=3.23 at q=0) near the inward rectifying potassium channel gene KCNJ2 encoding the Kir2.1 potassium channel. A missence mutation in KCNJ2 (encoding D71V) was identified in the linked family. Eleven additional mutations were identified in unrelated patients. Expression of these mutations in Xenopus oocytes revealed loss of function and a dominant negative effect in Kir2.1 current as assayed by voltage-clamp. We conclude that mutations in Kir2.1 cause Andersen's syndrome. These findings suggest that Kir2.1 plays an important role in developmental signaling in addition to its previously recognized function in controlling cell excitability in skeletal muscle and heart. Ongoing work is being directed at understanding the transcriptional regulation of KCNJ2 in development and the role of mutations in causing the features of AS.

Program Nr: 276 from the 2001 ASHG Annual Meeting

Mutations in KCNJ2, an inward rectifier potassium channel gene, cause Andersen's syndrome. *I. Splawski[1,2], K.W. Timothy[5], S. Priori[6], P.J. Schwartz[7], M.T. Keating[1,3,4].* 1) Department of Cardiology, Children's Hosp, Boston, MA; 2) Department of Pediatrics, Harvard Medical School; 3) Department of Cell Biology, Harvard Medical School; 4) Howard Hughes Medical Institute; 5) Department of Human Genetics, University of Utah, Salt Lake City, UT; 6) Molecular Cardiology, Fondazione Salvatore Maugeri, Pavia, Italy; 7) Department of Cardiology, Policlinico S. Matteo IRCCS and University of Pavia, Pavia, Italy.

Andersen's syndrome (AS) is a disorder characterized by potassium-sensitive periodic paralysis, cardiac arrhythmias, and dysmorphic features. The dysmorphology includes short stature, low set ears, broad nose and forehead, mandibular hypoplasia (small chin), scoliosis (lateral curvature of the spine), clinodactyly (curved finger or toe), and syndactyly (webbing) of the toes. AS is inherited as an autosomal dominant trait but the phenotypic characteristics of AS individuals are quite variable even within the members of the same family. KCNJ2 encodes the inward rectifying potassium channel Kir2.1. KCNJ2 expression is detected in multiple tissues, showing highest levels in heart, skeletal muscle, and brain. We used sequencing analysis to screen KCNJ2 in several unrelated probands with AS. Three missense mutations were identified in probands from two small families and a sporadic case - Tyr68Asp, Asp78Tyr, and Asn216His. All three mutations affected important functional domains of Kir2.1. The mutations were present in all affected individuals of the respective families. These mutations were not found in unaffected family members and in 100 control individuals (200 chromosomes). Mutations in KCNJ2 were not detected in several Andersen's syndrome families, suggesting the existence of additional gene(s) responsible for this disorder. In summary, mutations in the Kir2.1 gene, KCNJ2, are a major cause of the phenotypic features associated with Andersen's syndrome.

Program Nr: 277 from the 2001 ASHG Annual Meeting

Three-dimensional multicolor FISH for the single cell analysis of tumor interphase nuclei. *M.R. Speicher[1], J. Kraus[1], A. Bolzer[1], R. Gangnus[1], C. Maierhofer[1], I. Jentsch[1], S. Langer[1], G. Lederer[1], C. Keri[1], C. Fauth[1], K. Saracoglu[2], R. Eils[2]*. 1) Institute of Human Genetics, TU Munchen, Munchen, Germany; 2) Deutsches Krebsforschungszentrum, Heidelberg, Germany.

We have developed new strategies for multicolor interphase-FISH. Seven different fluorochromes are used for the simultaneous visualization of up to 24 region-specific probes (YACs, BACs, PACs, cosmids, painting probes). Images are captured using an epifluorescence microscope equipped with a motorized table to collect a stack of images at defined levels in z-direction. In a first step images are processed with deconvolution to remove out-of-focus information. In a second step combinatorially labeled probes are classified in an automated fashion. To achieve this the automated color classification used for multiplex-FISH (M-FISH) was extended by combining both color and local neighborhood information. Application of this new software allows the analysis even of noise-dominated images. In a third step, 3D-reconstruction algorithms are applied. Diagnostic applications include the simultaneous hybridization of 13 region specific probes covering regions often lost or gained or involved in translocations in B-CLL. In addition, this approach was applied on thick tissue sections of breast and ovarian tissue samples in order to study the heterogeneity in these tumors. Moreover, to study the three-dimensional organization of the genome our M-FISH mix was hybridized to cell nuclei with subsequent reconstruction of all chromosome domains in different colors.

Program Nr: 278 from the 2001 ASHG Annual Meeting

DOP-PCR based array-CGH for extensive amplicon profiling of breast cancers: A new approach for the molecular analysis of paraffin-embedded cancer tissue. *Y. Daigo[1], S-F. Chin[1], G. Callagy[1,2], K. Gorringe[1], G. Iyer[1], L. Bobrow[2], J.D. Brenton[1], P. Pharoah[1], C. Caldas[1]*. 1) Dept Oncology; 2) Dept Pathology, Univ Cambridge, Cambridge, England.

The development of high throughput technologies has opened the way for whole genome analysis of cancers. Until recently these analyses could only be carried out on fresh tissue and required significant amounts of scarce tissue resources, whereas, paraffin-embedded tissues are routinely available. Hence, we aimed to establish the validity of DOP-PCR, such that the array-CGH analysis of large series of microdissected cancer lesions becomes possible. Array-CGH was performed following a protocol modified to use DOP-PCR product. Test DNA and reference DNA labeled by DOP-PCR with a green/red fluorophore, were hybridized to Vysis AmpliOnc I DNA array (57 oncogenes). Arrays were analyzed using Vysis GenoSensor System. An excellent correlation was observed between array-CGH, using the two different labeling methods (DOP-PCR/nick-translation) and FISH analysis in 9 cell lines and 2 tumors using 4 genes as probes (100% sensitivity/specificity). Comparison of copy number changes detected by DOP-PCR based array-CGH in 20 breast cancers to those detected by conventional array-CGH, demonstrated that amplifications can be reliably detected (Spearmans $r=0.68$, $p<0.001$; 90.6% sensitivity and 99.6% specificity). The result of DOP-PCR based array-CGH in two laser microdissected paraffin-embedded cancers showed significant concordance with that of nick-translation based method in frozen tumors. We screened 96 breast cancers and obtained a detailed amplicon profile that is the most comprehensive to date in breast cancer. In all, 1031 gains covering 49 genetic loci from 22 chromosomes were observed. We also compared in 35 breast cancers amplifications of genes detected by array-CGH with data on chromosomal amplicon obtained by CGH. In most cases with amplicons detected by CGH there was a significant number with amplification of oncogenes known to be within the amplicon. The array-CGH method developed here will allow the genetic analysis of paraffin-embedded cancer materials for example in the context of clinical trials.

Program Nr: 279 from the 2001 ASHG Annual Meeting

Association of Cri du Chat Phenotype with DNA copy number abnormalities measured by array CGH. *X.. Zhang[1,2,4], E. Niebuhr[3], A. Snijders[1], R. Segraves[1], G. Hamilton[1], N. Brown[1], S. Blackwood[1], K. Hindle[1], A. Niebuhr[3], Z. Xiuqing[2], J. Gray[1], H. Yang[4], D. Albertson[1], L. Bolund[2], D. Pinkel[1]*. 1) UCSF, San Francisco, CA; 2) Aarhus Univ, Aarhus Denmark; 3) Copenhagen Univ, Copenhagen Denmark; 4) Institute of Genetics, Beijing, China.

Cri du Chat syndrome, cytogenetically characterized by deletions on chromosome 5p, consists of a variable phenotype that includes dysmorphic facial features, a characteristic cry, and mental retardation. Previous studies have roughly associated various aspects of the phenotype with the extent of the deleted region. We have analyzed 98 Cri du Chat cases using array CGH (Pinkel et al. Nat Gen 20:207-211, Albertson et al. Nat Gen 25:144-146, Cheung et al. Nature 459:953-958) with a set of approximately 100 BAC, PAC, P1 and cosmid clones distributed along chromosome 5p, as well as control clones at other locations. Detailed phenotypic information, including degree of mental retardation was available for each of these individuals. Measurement precision is such that single copy gains and losses affecting single clones are reliably detected, permitting accurate mapping of the boundaries of deletions and detecting additional copy number alterations that occurred in a significant fraction of the cases. Approximately 75% of the cases had a terminal deletion of 5p, 13% an interstitial deletion, and 4% a deletion plus a small duplication at the deletion boundary. In addition, approximately 8% had more complex aberrations involving other chromosomes that were detected using another array of ~ 2400 BACs that allowed scanning the entire human genome with about 1.5 Mb resolution (Snijders et al. Am J Hum Genet 67 sup 2: 31). We have provisionally located the cry and facial features to a minimal common region of 1.5 Mb at 5p15.3, speech delay to a 9 cM region at 5p15, and determined the dependence of mental impairment on the details of the aberration. Aberrations on chromosomes in addition to 5 may contribute to the phenotype in a significant portion of the cases. Supported by R01-HD17665, R01-CA83040, U01-CA84118, Vysis Inc.

Program Nr: 280 from the 2001 ASHG Annual Meeting

Screening for copy number variation in subtelomeric DNA by fluorescent Multiplex Amplifiable Probe Hybridisation. *E.J. Hollox[1], T. Atia[2], T. Parkin[2], J.A.L. Armour[1]*. 1) Institute of Genetics, University of Nottingham, Nottingham, UK; 2) Centre for Medical Genetics, City Hospital, Hucknall Road, Nottingham, UK.

Sub-microscopic copy number changes of subtelomeric regions of the human genome have been implicated in the etiology of several clinical conditions, including mental retardation. Fluorescent in-situ hybridisation (FISH) using a set of subtelomeric probes has been used for screening these regions, but this is expensive and can be laborious, imposing a practical limit on the numbers of patients who can be screened. We have used Multiplex Amplifiable Probe Hybridisation (MAPH) to develop a screen for DNA copy number at subtelomeric regions. We have constructed a set of probes with each subtelomeric region represented at least once, except the p ends of acrocentric chromosomes. Each probe has been sequenced and its position relative to the telomere determined. We have used fluorescent detection on a Pharmacia ALF machine and an ABI 377 DNA sequencer, both platforms that are commonly used in diagnostic and research laboratories. This implementation allows a high throughput of samples to be achieved compared to FISH, with analysis that could potentially be automated.

The set of probes has been characterised on 183 phenotypically normal individuals to provide reference data on polymorphism and experimental variation. We have also characterised most probes on patients/cell lines with known subtelomeric changes. To test the suitability of the method for clinical applications, we have screened 42 DNA samples initially referred for FRAXA testing, and found at least one with an abnormality (duplication of 3p) detected independently by our two laboratories. Results can be tested statistically, and a suitable significance threshold can be used to minimise the number of false negatives and keep the number of false positives to an acceptable level. Therefore this method can be simply implemented as a first round high-throughput screening technology, using established laboratory equipment, to identify selected individuals and loci for further study by FISH.

Program Nr: 281 from the 2001 ASHG Annual Meeting

Molecular Mechanisms of Terminal Deletions. *B.C. Ballif, H.A. Heilstedt, C. Knox-Du Bois, L.G. Shaffer.* Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

A better understanding of the mechanisms which result in and stabilize cytogenetically defined terminal deletions may be extremely valuable since the majority of all cytogenetically visible deletions involve the distal, telomeric bands of the chromosomes. Terminal deletions of 1p36 are of particular interest since they result in a mental retardation syndrome that occurs in roughly 1 in 5,000 live births making it the most common terminal deletion. Although the mechanisms by which terminal deletions arise and are stabilized are not completely understood, all ends of human chromosomes must have a telomeric cap to be structurally stable. Chromosome analysis of 50 patients with cytogenetically visible deletions of 1p36 using telomere region-specific FISH probes revealed 5 (10%) cases to be interstitial deletions that retained the 1p telomere, 9 (18%) cases to be derivative chromosomes that acquired a different chromosome end (7 of 9 cases were de novo), and 36 (72%) cases to be apparently pure terminal deletions. This suggests that terminal deletions of 1p36 are formed and stabilized by a variety of mechanisms, although we speculate that the majority of cases were stabilized through telomere regeneration. Microsatellite and FISH analyses indicate that these deletions are variable in size. Somatic cell hybrids carrying only the deleted chromosome 1 have been generated for four cases of apparently pure terminal deletions, and the systematic amplification of STS markers from DNA extracted from 2 of these hybrid cell lines has allowed us to map their precise breakpoints to within 1 kb regions. Although both of these breakpoints lie in the most repeat-rich regions of their respective 150 kb surrounding areas, the precise breaks appear to have occurred within non-repetitive, unique sequences. Aside from an abundance of various repetitive elements, there is no sequence homology between these 2 breakpoint regions. We speculate that these chromosomes were "healed" by telomere regeneration, and we are comparing these breakpoints with those of other terminal deletions of different chromosomes stabilized by de novo addition of telomeric repeats.

Program Nr: 282 from the 2001 ASHG Annual Meeting

Elucidation of the mechanisms of chromosome rearrangement: Implications for heightened complexity. *C. Astbury[1], L. Christ[1], A. Kumar[2], C. Crowe[3], S. Schwartz[1]*. 1) Case Western Reserve Univ & Univ Hosp of Cleveland, Cleveland, OH; 2) Ind Inst Science, Bangalore, India; 3) Metro Health Med Ctr, Cleveland, OH.

It has always been assumed that chromosome rearrangements form by means of the simplest mechanism and the fewest number of breaks. Recent studies have revealed that some terminal deletions are in fact interstitial and some rearrangements are more complex than originally believed. In this study, we have surveyed a large number of rearrangements to determine their complexity, by utilizing high-resolution chromosomes and FISH. The approach was to look at various rearrangements and apply molecular cytogenetics to determine the mechanism of formation and actual number of breaks in the rearrangement. Our results yield a number of significant findings: (1) In an overall survey of terminal deletions 44/107 cases (41%) were more complex than expected, involving either duplications or inversions. Additionally, 14/18 (78%) of de novo translocations, ascertained in phenotypically abnormal individuals, involved more than 2 breaks. (2) Eight apparently balanced de novo rearrangements in individuals with multiple anomalies were studied with BACs and YACs, and 7 were shown to have cryptic deletions. This reinforces our previous work that this mechanism may be responsible for the majority of phenotypic abnormalities seen in de novo rearrangements. (3) Two families had unexpected cryptic terminal rearrangements in addition to an obvious inversion and translocation in each case. (4) Two probands had multiple rearrangements (with >10 breaks). Results from these studies are important in that they provide additional insight into the mechanism of constitutional abnormalities. We can conclude that: (1) rearrangements involving 3 breaks are more common than originally envisioned; (2) a higher frequency of de novo derivative chromosomes with both deletion and duplications have been illustrated by high-resolution chromosomes and molecular analysis; (3) deletion of chromosomal material in de novo rearrangements accounts for a large proportion of cases with phenotypic abnormalities; and (4) cryptic telomeric involvement is important to detect as they will account for alterations in segregation ratios.

Program Nr: 283 from the 2001 ASHG Annual Meeting

FISH studies in patients with aniridia reveals >35% with chromosome abnormalities including five cryptic 3 PAX6 deletions. *J.A. Crolla[1], V. van Heyningen[2].* 1) Wessex Reg Genetics Lab, Salisbury District Hosp, Wiltshire, England; 2) MRC Human Genetics Unit, Western General Hospital Edinburgh EH4 2XU.

Seventy patients with aniridia (13 familial and 57 sporadic), referred for FISH and cytogenetic investigations, were studied initially using cosmids mapping to distal 11p13, (cen B2.1(WT1), D11S324, FAT5 {PAX6 exons 1-4}, and FO2121, tel). Finding a case with a deletion not involving FAT5 but including the marker FO2121, ~100kb telomeric, prompted additional studies on all cases reported as normal by FISH. A cosmid contig, mapping to a 180kb region 3` of PAX6, was used: (cen A1280 {PAX6 exons 5-13}, G0453, C1170, H1281, SRL11M20, SRL9A13, tel). Five of the 48 normal cases re-tested (7% of all cases) had small deletions ~50-430kb in size, all with a similar proximal breakpoint in cosmid A1280, indicated by diminished signal on one chromosome 11. One case with an ~180kb deletion was mosaic, with half the peripheral blood lymphocytes with the deletion and half with normal signals on both 11 homologues. Overall, 27 cases (38.6%) were chromosomally abnormal. Twelve (17%) had cytogenetically visible interstitial WAGR deletions involving 11p13, ten of which included WT1. Eleven (15.5%), including the five described above, had cryptic deletions only visible by FISH, two include WT1. The remaining four cases (5.5%) had chromosomal rearrangements: an unbalanced t(11;13) with a deletion of the WAGR region, and three (4%) balanced rearrangements with what appear to be position effect breakpoints 3 of PAX6; (a) an inv(11)(p13q13) with a breakpoint in cosmid H1281 (>75kb downstream of PAX6) (b) a t(7;11) with the 11p13 breakpoint ~30kb downstream of PAX6 and (c) a dir ins(12;11) with a breakpoint in cosmid C1170 (>40kb from PAX6). The rates and distribution of chromosome anomalies in familial (4/13, 30.8%) and sporadic cases (23/57, 40%) are similar. Whether the reduced A1280 signal indicates that the PAX6 gene is disrupted, remains to be assessed. If the 13 PAX6 exons are intact, these cases represent interstitial deletions resulting in position effects. As the five small deletions were not identified using cosmid FAT5, A1280 should be incorporated into diagnostic FISH procedures.

Program Nr: 284 from the 2001 ASHG Annual Meeting

Dissection of the 11q13 amplicon in oral cancer cells. *X. Huang[1], T.E. Godfrey[1,2,3], S.M. Gollin[1,3,4]*. 1) Department of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; 2) Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA; 3) University of Pittsburgh Cancer Institute, Pittsburgh, PA; 4) Oral Cancer Center at the University of Pittsburgh, Pittsburgh, PA.

Chromosomal instability is an important feature of cancer cells. Gene amplification is one form of chromosomal instability. It is also a genetic mechanism for producing a higher level of gene product. About half of oral squamous cell carcinomas (OSCC) and derived cell lines express amplification of chromosomal band 11q13 in the form of a homogeneously staining region. We and others have proposed that 11q13 gene amplification occurs via sister chromatid fusion followed by breakage-fusion-bridge (BFB) cycles. We have used quantitative microsatellite analysis (QuMA) to map the proximal and distal breakpoints of the amplicon. In the 20 OSCC cell lines with 11q13 amplification examined to date, the core of the amplicon has been identified as a 400kb region. The proximal breakpoints in 11 of 20 cell lines (56%) map to two adjacent breakpoint intervals; one is 550kb in length and the other is 160kb. This finding suggests the presence of one or two as yet unidentified breakage hotspots or fragile sites that appear to be responsible for the proximal breakpoints of the 11q13 amplicon. The distal breakpoint maps to one interval in 4 of the 20 cell lines (20%). These results also support the sister chromatid fusion/BFB model for 11q13 amplification. In contrast to what we expected, the copy number of the *CCND1* gene is not the highest in the 11q13 amplicon. We identified one interval that was amplified to a significantly higher copy number in the core of the 11q13 amplicon, in which new ESTs are located. This result may indicate that new genes corresponding to the ESTs may play a significant role in driving 11q13 amplification and in the development and progression of oral cancer. [Supported in part by NIH grants R01DE12008, P60DE13059, and P30CA47904.].

Program Nr: 285 from the 2001 ASHG Annual Meeting

The significance of trisomy 8 in myeloid leukemia: a Southwest Oncology Group (SWOG) study. *M.L. Slovak[1], H. Gundacker[2], S.R. Wolman[3], F.R. Appelbaum[4] for the Southwest Oncology Group, San Antonio, TX.* 1) Department of Cytogenetics, City of Hope National Medical Center, Duarte, CA; 2) Southwest Oncology Group Statistical Center, Fred Hutchinson Cancer Research Center, Seattle, WA; 3) Wayne State University, Detroit, MI and UAREP, 9650 Rockville Pike, Bethesda, MD; 4) Fred Hutchinson Cancer Research Center, Seattle, WA.

The prognostic impact of trisomy 8, as the sole aberration or with other clonal aberrations, was evaluated in 928 patients registered to six Southwest Oncology Group trials. At presentation, 118 patients (12.7%) had +8 including 43 (4.6%) cases with +8 as sole aberration, 307 (33.1%) had normal karyotypes, and 503 (54.2%) had karyotypes with other clonal cytogenetic abnormalities. When patients with (n = 118) and without +8 (n = 810) were compared, +8 patients were slightly older (p = 0.043), had lower WBC (p = 0.026), and had considerably lower percentages of peripheral blasts (p = 0.0003). Although no differences were observed in disease free survival (logrank p = 0.63), median survival for all patients with +8 was 8 months (mo), a finding equivalent to reported "unfavorable" cytogenetics risk groups. When compared to patients with normal cytogenetics, patients with +8 had significantly lower peripheral blasts, WBC counts and decreased overall survival (median, 15 mo vs 8 mo, respectively), whether studies were combined (p=0.0007) or stratified by study (p=0.0007). However, survival of patients with +8 as the sole aberration did not differ significantly from those with normal cytogenetics (12 mo vs 15 mo, respectively) (p>0.36). Furthermore, the small numbers of patients with t(15;17) with or without +8 at presentation did not differ with respect to any of the clinical-pathologic parameters examined. Thus, although the +8 group as a whole had poor median survival, survival differences were attributable to increasing complexity of cytogenetic aberration rather than to trisomy 8 per se.

Program Nr: 286 from the 2001 ASHG Annual Meeting

Trisomy recurrence: A reconsideration based on North American data. *D. Warburton[1], J. Garniez[1], L. Dallaire[2], M. Thangavelu[3], L. Ross[3], D. Anaya[3], C. Pargas[3], J. Kline[4].* 1) Columbia Univ, New York, NY; 2) Ste-Justine Hospital, Montreal, Canada; 3) Genzyme Genetics, Orange, CA and Santa Fe, NM; 4) New York State Psychiatric Institute, New York, NY.

Despite the large numbers of prenatal diagnoses performed for detection of trisomy, there are few published data on trisomy recurrence risk. In particular, it is unknown whether a woman who has had a conception with one trisomy is at increased risk for trisomy of a different chromosome. Recurrence risks for trisomy 21 are most often based on an analysis of European amniocentesis data from the 1980s. There were not enough data to determine recurrence risks for other trisomies. Analysis of women with two karyotyped spontaneous abortions, however, suggested that women with trisomic spontaneous abortions were not at increased risk for a subsequent trisomic conception. The question of whether or not some women are at increased risk for trisomy in general bears on several hypotheses about the origins of trisomy: e.g. a generalized increase would be predicted by the limited oocyte pool model concerning the maternal age effect, and by genetic variation in recombination patterns affecting nondisjunction. We analyzed data from two new sources: Genzyme Genetics in the USA (1994 to 2001), and Ste-Justine Hospital in Montreal, Canada (1976-1999). Maternal ages were available at both the first and second pregnancies for 1387 prenatal diagnoses (1021 women) subsequent to a pregnancy with trisomy 21, and for 884 prenatal diagnoses (832 women) subsequent to a trisomy other than 21. We compared the observed number of trisomies with that expected based on risk at amniocentesis or CVS by single year of maternal age. In contrast to the analysis of the European data, risk of a subsequent trisomy 21 was increased not only for women with their first trisomy at £30 years but also for those with a first trisomy at >30 years. There was also a moderately increased risk for other non-lethal trisomies, both among women with a previous trisomy 21 pregnancy and those with a previous lethal trisomy ending in spontaneous abortion. These data suggest that a reconsideration of the counseling in such cases may be necessary.